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14. ABSTRACT
Prostate cancer (PC) is the second leading cause of male U.S. cancer deaths, with African-Americans having the highest rate of PC mortality worldwide, as well as more abnormal results from screening tests that correlate with current or eventual PC. A 5-year prospective NIH-funded clinic-based study investigated whether prostate-specific antigen (PSA) and digital rectal exam (DRE) screening indicators of PC risk in 500 African-American men 50 to 70 years of age who underwent PC screening in Oakland, CA (East Bay San Francisco area), were associated with estimated dietary exposures to 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), which forms when meat is overcooked. The DOD-funded study expands that NIH-funded work by adding a new %-free-PSA test for 310 (108 from the NIH-funded study, plus 202 additional) men, results of which will be compared with PSA/ DRE results and PhIP exposures estimated by dietary interviews. For 392 men studied under the NIH protocol, an odds ratio (95% CL) of 32 (3.2, 720) for highly elevated PSA (≥20 ng/mL) was observed in the highest 15% vs. the lower 50% of estimated daily PhIP intakes. As of 09-01-07, a total of 310 men completed participation using the expanded protocol, for a combined total of 702 men. For the final analysis of all 702 men, the corresponding OR was found to be 10 (2.9, 58). This study will help define the potential value of improved screening and dietary/behavioral intervention to reduce PC risk.

15. SUBJECT TERMS
African-American men, cooked-meat carcinogens, epidemiology, PhIP, prostate cancer, screening

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Introduction

PhIP is a Dietary Carcinogen that May Pose Heightened Risk to African-American Men

African American (AA) men, who compared to Caucasians die nearly twice as much from prostate cancer (PC), also take in about twice as much of the predominant U.S. dietary heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Bogen and Keating, 2001), which occurs primarily in well-cooked chicken and beef. Heterocyclic amines (HAs) are potent mutagens formed in meats, chicken and fish as it is cooked to higher-doneness levels by heat-intensive cooking methods (Thompson *et al*., 1987; Keating et al., 1999, 2000). HAs also cause cancer at a variety of sites in multiple bioassay animal species/strains/sexes, as well as at multiple sites within many of species/strains/sexes tested (Bogen, 1994). A predominant HA found in cooked and particularly in well-done chicken and beef is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Felton et al., 1984, 1986; Felton and Knize, 1990a-b; Sinha et al., 1995). Dietary exposure to PhIP has been shown to induce colon, intestinal and mammary adenocarcinomas in rats (Ohgaki et al., 1986; Ochiai et al., 1991; Ito et al., 1991; Ito et al., 1997; Ghoshal et al., 1994), as well as prostate cancer in rats (Shirai et al., 1997,1999). HAs are metabolically activated by P450 and N-acetyltransferase enzymes to activated forms that bind covalently to (among other targets) DNA in tissues (including in prostate) where HAs induce cancer in rats (Thorgeirsson et al., 1983; Thorgeirsson, 1984; Rosenkranz and Mermeistein, 1985; Sato et al., 1986; Kato and Yamazoe, 1987; Snyderwine and Battula, 1989; McManus et al., 1990; Turesky et al., 1991; Davis et al., 1993; Kaderlik et al., 1994a-b; Takahashi et al., 1998; Schut and Snyderwine, 1999; Gooderham et al., 2002). In male *lacI* trangenic rats, a diet containing 200 ppm PhIP was shown to be highly mutagenic in prostate tissue (Stuart *et al*., 2000). In cultured human prostate tissue and primary prostate cells, PhIP is metabolically activated to mutagenic forms that covalently bind to and damage DNA (Williams et al., 2000; Lawson and Kolar, 2002; Martin et al., 2002; Di Paolo et al., 2005).

PC and African-American men

In the U.S., prostate cancer (PC) is a leading cause of cancer death among men, with African Americans having the highest age-specific prostate cancer rate in the world, and a >2-fold higher rate of mortality for PC than white men in the U.S.(Miller *et al*., 1996; Robbins *et al*., 1998; Hsing *et al*., 2000). Although family (particularly father/brother) history of PC is clearly linked to substantially elevated PC risk (Schuurman *et al*., 1999; Hemminki and Czene, 2002; Zeegers et al., 2003; Hemminki and Chen, 2005) and a human-PC-specific chromosome translocation has been identified (Tomlins et al., 2005), there is (as for most other cancers) no evidence for a predominant heritable factor for PC. Racial-ethnic differences in levels of testosterone-related hormones and related genetic controls on hormone-induced prostate growth have been hypothesized to explain or contribute to corresponding racial-ethnic differences in PC risk, but their role remains unclear (Ross *et al*., 1998; Pettaway, 1999; Hsing, 2001). Even if hormonally mediated background processes do affect PC risk, dietary exposures to genotoxic HA carcinogens may act independently or interact synergistically with such hormonal processes to further modify PC risk.

HA, Meat, Other Dietary Factors, and PC Risk

Substantial geographical variations in PC incidence indicate the importance of one or more dietary or other environmental factors (Minami et al., 1993; Mettlin, 1997; Angwafo, 1999). Dietary intake of animal or saturated fat is the environmental factor

most consistently linked to significantly increased PC risk in previous studies, particularly among African-American men, though these associations appear too weak to explain more than a small fraction of observed racial-ethnic differences in PC risk (Whittemore *et al*., 1995; Hayes *et al*., 1999; Kolonel *et al*., 1999; Daniels et al., 2004), as also appears to be the case for other environmental/dietary factors examined such calcium, cruciferous vegetables, vitamin D, UV from sunlight, lycopene, and body size (Giovannucci *et al*., 1997, 1998; Cohen *et al*., 2000; Chan and Giovannucci, 2001a-b; Kristal and Lampe, 2002; Bodiwala et al., 2003). Because cooked-meat intake is positively associated with total saturated-fat intake (USDA, 1998), previous studies that focused on animal or saturated fat *per se* could have estimated effects due largely or entirely to meat-related HA intakes.

Consumption of cooked meats and associated HAs have been linked to increased risks of colorectal adenoma/adenocarcinoma and of cancer of the stomach, breast, lung and prostate (Mills *et al*., 1989; Shiffman and Felton, 1990; Gerhardsson de Verdier *et al*., 1991; Talamini *et al*., 1992; Lang *et al*., 1994; De Stefani *et al*., 1995; Ewings and Bowie, 1996; Probst-Hensch *et al*., 1997; Ward *et al*., 1997; Kampman *et al*., 1999; Norrish *et al*., 1999; Sinha *et al*., 1998a, 1999a-b; Zheng *et al*., 1998, 1999; Murtaugh et al., 2004), while fewer studies found no such associations (Lyon and Mahoney, 1988; Muscat and Wynder, 1994; Augustsson *et al*., 1999). Positive studies include those in which HA exposure was quantified adjusting for factors expected to determine HA intake—namely, meat type, consumption frequency, cooking method, and cooking doneness. A potential link between PhIP intake, in particular, and the elevated risk of PC experienced by African-American compared to Caucasian men is suggested by relatively greater levels of PhIP and its metabolites detected in urine sampled from the latter vs. the former group (Kidd *et al*., 1999), although urine analysis only provides a measure of PhIP exposure within 12-24 hours prior to sampling (Malfatti *et al*., 1999). Such a link is also supported by studies using meat- and cooking-method-specific HA-concentration estimates from multi-laboratory sets of experimental cooking data (Keating and Bogen, 2001) to assess intakes of PhIP and other HAs by >20,000 U.S. (including >3,000 African-American) participants in the nationwide, stratified, random-sample U.S. Continuing Survey of Food Intakes by Individuals (CSFII) (USDA, 1993, 1998). Analyzed by age-, sex, and race/ethnicity, these HA-exposure assessments found at all ages that African-American males consume on average at least twice as much PhIP (and total HA) per kg body weight per day as do U.S. Caucasian males, and that for both groups there is a significant positive (albeit small) correlation between estimated mean intakes of PhIP and those of saturated or total fat (Keating and Bogen, 2001; Bogen and Keating, 2001).

Although the study comparing HA intake (primarily from cooked lamb) and PC risk in New Zeeland men by Norrish *et al*. (1999) found no significant PhIP-related associations involving PC, that study did report a significant association between PC risk and consumption of beefsteak by higher cooking-doneness category (2-sided p_{trend} = 0.008). Several aspects of that study suggest it may have had limited power to assess potential associations between PC and HA or PhIP intake in the U.S. The incidence of PC in New Zeeland is only half that of Caucasian men in the U.S. (Hsing et al. 2000). Average meat (primarily lamb) consumption by subjects in the Norrish et al. study (~150 g/d) was well below that of U.S. Caucasian men (260 g/d) and very well below that of African-American men (304 g/d) (USDA, 1998; Table 10A). Moreover, the *minimum* PhIP intake (224 ng/d) in the *top* exposure quartile of Norrish et al. study subjects was

well below the estimated *average* PhIP intakes by U.S. Caucasian (390 ng/d) and by African-American (600 ng/d) men (Bogen and Keating 2001).

A randomized, controlled multisite prospective study that ascertained 1,338 PC cases between 1993 and 2001 among 29,361 (including 3.3% African American) men of age 55-74 years in 10 U.S. cities was conducted specifically to determine whether meat intake or meat-related mutagens, including PhIP, was associated with increased PC risk (Cross et al., 2005). In this study, intakes of meat, PhIP, two other heterocyclic amines, and meat-associated Ames-assay mutagenic yield were all estimated using FFQs that were self-administered, but otherwise very similar to those currently administered (by in-person interview) in the clinical study which Project 2 of this application would extend. More than 10 g/day vs. no intake of very well done meat was found to be associated with a 1.7-fold increased risk (95% CI: 1.19-2.40), and the highest vs. lowest PhIP quintile with a 1.3-fold increased risk (95% CI: 1.01-1.61), of PC incidence (Cross et al., 2005). Other intakes were not found to be associated with increased PC risk. No separate analysis was done of study results by race-ethnicity was not reported, nor were genotype or other (e.g., dietary) factors considered that are known to affect PhIP metabolism.

PC Screening and PC

Periodic screening for plasma levels of Prostate-Specific Antigen (PSA) is useful for early detection of PC as well as benign prostatic hyperplasia (BPH) because PSA levels in serum are positively associated with age, prostate volume, and prostatic neoplastic disease (Carter *et al*., 1992; Oesterling *et al*., 1993; Etzioni *et al*., 1999). Significantly higher PSA levels are found in African-Americans than in whites, even after adjustment for age and prostate volume (in men without PC) and for PC grade and stage (in men with PC) (Abdalla *et al*., 1998a-b; Vijayakumar *et al*., 1998), due evidently to greater PSA secretion per unit prostate volume by African-American men (Fowler et al., 1999). Although a substantial fraction of “slightly” elevated PSA levels (between 4 and 10 ng/mL) is attributable to BPH or infection, “highly elevated” PSA levels (≥20 ng/mL) typically indicate a strong likelihood of localized or metastatic PC, with 80 to 90% positive predictivity and >99% specificity (Määttänen et al., 2001; Gerstenbluth et al., 2002; Smith et al., 2004). Likewise, a PSA measure < 4.0 ng/mL often considered within the “normal” range is actually associated with about at 15% of later PC diagnosis (Thompson et al., 2004).

Report Body

Body

Objective/Hypotheses: The study goal is to broaden the scope and power of a ground-breaking study of potential association between dietary HA exposure and screening indicators of PC risk in African-American men by adding a newer %fPSA test to the PSA/DRE protocol now being used. We hypothesize 1) that the added %fPSA test will increase PC detection in our study population, and 2) that the combined screening, follow-up diagnostic and dietary survey data obtained will reveal a positive association between estimated HA intake and screening and diagnostic indicators of increased PC risk in our African American study population.

Specific aims: Our aims are to 1) Add a newer %fPSA test to PSA and DRE screens being done in a current study of potential associations between HA and PC in Oakland,

California, area African-American men. 2) Assess potential increased rate of PC identified by including the %fPSA test with PSA and DRE results in light of clinical follow-up diagnoses obtained. 3) Use PSA-related and DRE test results, together with corresponding follow-up diagnostic and dietary survey data, to assess the potential association of HA-related exposure factors and increased PC risk in African-Americans.

Study Design: This is a prospective, clinic-based screening study. For aim 1, 392 participants were solicited from an already-established network of churches, clinics and additional African-American community groups in the Oakland, CA, area. The DOD-funded work continues this effort, adding the new %fPSA biomarker. Detailed data on diet and meat consumption continue to be obtained by in-person interviews using established questionnaires, each followed by PSA-related and DRE screening tests, and follow-up diagnosis—a study design that avoids potential bias due to prior participant/investigator knowledge of PC status. Aims 2 and 3 are being accomplished by statistical data analysis, with aim 3 to be supported by incorporation of similar (other than %fPSA) data obtained for the 392 participants already obtained through the NIH-funded study that ended in 2006, for a total combined study size of 702 men. Our focus on African-Americans provides needed study power, in view of the greater PC risk faced by this specific group.

Study Extension: Study goals and reported outcomes were completed by September 2007. At this time the Principal investigator at LLNL (Bogen) left his position without filing the third Annual Report and the Final Report for the project. Dr, Garrett Keating, a co-investigator on the project, took on the role of Principal Investigator. Due to Dr. Keating's obligations to other research projects and ongoing outreach efforts at ABSMC, a no-cost extension of the study deadline from January 1 2008 until June 30 2008 was requested. During this period Dr. Keating recorded 86 hours of effort to the project. His effort on the project during this time was mostly committed to close-out activities of the study. Dr. Keating conducted communications with the IRB offices at LLNL, DOD, UCSF and ABSMC to determine the actions necessary to complete the study protocol. For ABSMC, additional follow-up with subjects with suspicious PSA results was required and this delayed completion of the IRB protocols for the various institutions. Dr. Keating also initiated procedures for long-term archiving of the blood samples at LLNL. This activity required LLNL institutional approval, consolidation, de-identification, and inventory of the blood samples and transfer of the samples to a new facility at LLNL. During the extension period, UCSF submitted a request for additional funds due to an accounting error in the calculation of their institutional overhead charges. Resolution of this matter required communication with the UCSF co-investigators and with LLNL and UCSF business staff. Dr. Keating gave presentations about the study at the University of California – Davis Cancer Center and at ABSMC during the extension period. Dr. Keating also initiated a collaboration with a co-investigator at UCSF (Chan) that lead to a proposal submission to the Health Disparities Program of the 2008 DOD CDMRP (Appendix). The Annual Report for Year 3 and this Final Report for the project were submitted during the extension period.

Work Plan Summary

A total of 1060 men were scheduled for the study from September 2002 through May 2007 (Table 2, Appendix). Contacted by the outreach program, word of mouth or other means, men called into the clinic, were questioned about eligibility and, if qualified, scheduled for a date to come to the clinic. Many men did not attend their scheduled appointment and in most cases rescheduled. Table 2 also indicates the

number of participants identified with an abnormal prostate screening (either by PSA or DRE, or both in some cases

A total of 392 participants were accrued previously in the NIH-funded study through December 2004 using dietary interviews, a digital rectal exam (DRE), and a standard prostate serum antigen (PSA) blood test. The DOD Prostate Cancer Research Program support has added a second PSA-related test— the "percent-free PSA" (%fPSA) test—that was applied to 310 additional study participants through September of 2007, for whom blood samples were drawn for both a PSA and a %fPSA test and the dietary interview and DRE done, all at the study clinic in Oakland on the same day for each participant (Table 3, Appendix).

Delayed Receipt of Funds and HSRRB Approval of Human Subjects Protocol

Initiation of the study was delayed due to delayed receipt of DOD funds by Lawrence Livermore National Laboratory (LLNL). Due to contract language negotiations, funds arrived at LLNL approximately four months after the official start date of the project (Jan. 5, 2005). A further start-up delay occurred due to delayed receipt of U.S. Army Human Subjects Research Review Board (HSRRB) approval of the study human subjects protocol, which had already received approval by the other three Institutional Review Boards (IRBs) involved in this study (those of LLNL, the University of California San Francisco Medical School, and the Summit Alta Bates medical Center in Oakland, CA). A draft human subjects protocol was submitted to the HSRRB in December of 2004, but HSRRB approval was not obtained until mid-May of 2005.

The delayed start-up and extended IRB-related documentation required to start this project caused small, unanticipated shifts in LLNL labor relative to the original budget plan. The project resumed screening in June, 2005 and conducted monthly screenings until May, 2007.

Key Research Accomplishments

The NIH-funded P01 work that set the stage for the present expanded study has accomplished the two specific aims it sought to address. We successfully applied methods for estimating HA concentrations in cooked meats based on individually expressed data on meat-specific intakes, cooking methods and doneness preferences to estimate daily PhIP intakes, and we have found these intake estimates to be positively associated with screening indicators of highly elevated PC risk in a prospective clinic-based PC screening study involving 392 African-American men in the San Francisco East Bay area. The observed positive association, which was most significant among men 55 to 70 years of age ($p_{trend} = 0.00020$), remained statistically significant after adjustments for saturated fat intake, total energy intake and self-reported father/brother history of PC. These study findings were upheld in updated analyses that involved a total of 562 men (see attached 2007 AACR abstract) and 702 men (see attached 2007 DOD IMPaCT abstract) from the combined NIH- and DOD-supported work. The %fPSA test added additional strength to the association of PC risk and PhIP intake in men with highly elevated PSA (≥20 ng/mL). Insufficient follow-up diagnostic results were obtained from these men to conduct an analysis of PhIP intake and PC and improved sensitivity of the %fPSA test. These findings will continue to help define the potential value of improved screening and dietary/behavioral intervention to reduce PC risk, namely, prevention of PhIP intake by avoiding overcooked meats.

Task 1. Add %fPSA test to the standard PSA blood test and DRE being done in a current study protocol to assess potential HA/PC associations in Oakland-area African American men (Months 1-36). **Completed as of September 1, 2007.**

1.A. Implement combined PSA-test protocol (PSA + %fPSA) for a total of 310 African American men screened at the Summit Alta-Bates MCEPC clinic in Oakland, California (Months 4-36). The %fPSA, PSA and DRE procedures to be used are all clinical procedures now performed routinely at the study clinic (Markstein Cancer Education and Prevention Center, Alta Bates Summit Medical Center, Oakland, CA). **Initiated as of June 15, 2005; completed September 1, 2007.**

1.B. We will interview study subjects and edit dietary questionnaires for all study subjects, to include:

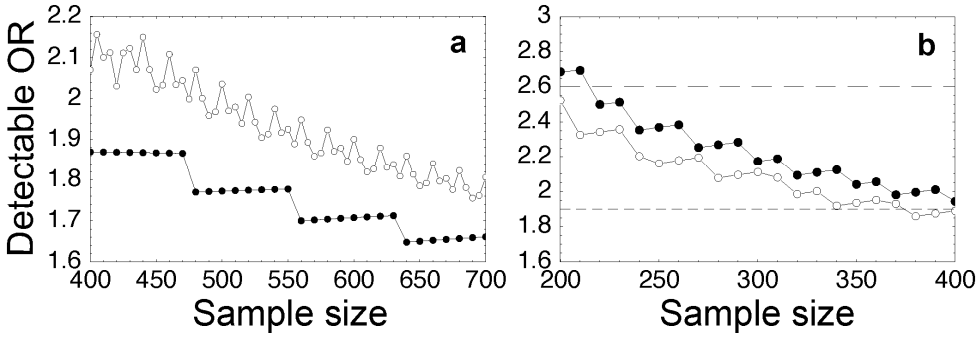
1.C.. For all participants to be screened in this study, previously developed dietary survey questionnaires will be used in the same manner they are being used in our ongoing corresponding NIH-funded research study (see Questionnaires, Surveys & Clinical Protocols). **Completed as of September 1, 2007.**

Task 2. Assess improvement in PC sensitivity and selectivity by addition of %fPSA test to the standard PSA test based on PSA-related test results and follow-up diagnoses in our African American study participants (Months 18-36). **Completed as of May 22, 2008.**

2.A. Data analysis methods to be used will be identical to those in use for the current related NIH-funded study (see Questionnaires, Surveys & Clinical Protocols)

2.B. Preliminary statistical analyses will be conducted during Months 18-27. **Completed as of December 31, 2006, except for %fPSA. See study publication attached.**

2.C. Final statistical analyses will be conducted during Months 27-36 **Completed as of May 22 , 2008. Insufficient numbers of PC cases were identified through clinic referral and follow-up to statistically evaluate the increased potential of the %fPSA test to detect PC. The figure below taken from the grant proposal indicates that a minimum of 5% PC detection in the study population (20 of 400) was required to evaluate the increased detection of PC with a %fPSA test in conjunction with the PSA test. Only 4 cases of PC were confirmed through clinical follow-up with participants for which %fPSA/PSA tests were obtained. Not all referrals were biopsied for PC. Twenty-two referrals were either lost to follow-up or refused to proceed to follow-up. The study population consisted of a significant proportion of men of low SES for who repeated follow-up became difficult as men changed their addresses or contact information.**



*Minimum odds ratio (OR) values detectable with 100(1-α)% = 95% confidence and 100P% power (P =1–β), as functions of sample size n. (**a**) Design in which P=80% and n = total sample size (number of study participants), assuming either that f = 20% of all tests are positive and P = 40% of those tested have relatively high HA intakes (solid points), or that f = 15% and P = 30% (open points). (**b**) Design to compare PSA vs. MSP test specificity where n = the total number tested by each method, PSA sensitivity = MSP sensitivity = 73%, and PSA specificity = 85%, assuming either that MSP specificity = s = 98%, f = 10% disease prevalence in those tested, and P = 95% (solid points—compare with expected OR = 2.6); or that s = 93%, f = 5% and P = 90% (open points—compare with expected OR = 1.9). Exact OR values plotted were obtained using Mathematica® 4.2 and related software (Wolfram, 1999; Bogen, 2002) to invert noncentral hypergeometric distributions evaluated under the stated assumptions (Zelterman, 1999) .*

Task 3. Use Combined PSA-related test results, together with dietary survey data, to assess the potential association of HA-related exposure factors and increased PC risk in African Americans (Months 13-36). **Completed as of September 1, 2007. See study abstracts attached.**

3.A. Data analysis methods to be used will be identical to those in use for the current related NIH-funded study.

3.B. Preliminary statistical analyses of the statistical validity of Study Hypotheses 1 and 2 will occur during Months 18-27. **See study publication attached.**

3.C. Final statistical analyses of the statistical validity of Study Hypotheses 1 and 2, as well as manuscript preparation, will occur during Months 27-36. **Completed as of September 1, 2007. See study abstracts attached. Specific Aim 1 of the study was to assess the potential of the %fPSA test to increase rate of PC identified by including the %fPSA test with PSA and DRE results in light of clinical follow-up diagnoses obtained. There was no association between %fPSA, PSA and PC diagnosis in the study, due in part to the low number of cancers ultimately detected through follow-up. No determination on the efficacy of the %fPSA test to improve PC detection can be made from the findings of this study.**

Analysis of initial data set (n = 392) obtained using the original protocol for NIH-funded work is described in the attached study publication. An updated analysis involving a total of 562 men was completed and was presented at the 2007 annual meeting of the American Association for Cancer Research (See attached 2007 AACR abstract). An final analysis involving a total of 702 men was completed and was presented at the 2007 DOD Congressionally Mandated Medical Research Program meeting, Innovative Minds in Prostate Cancer Today, September 5-8, 2007, Atlanta, GA (See attached 2007 DOD ImPACT abstract). The results of both analyses support the key study hypotheses. In the final analysis of 702 men (Table 1), an odds ratio, OR, (and maximum-likelihood 95% confidence limits) of

10. (2.9, 58) for men at high PC risk was observed in the highest quartile compared to the lowest half of estimated daily PhIP intakes (328.0 vs. 213.6 ng kg-1 d-1) (ptrend = 0.00005). This observation supports the principal hypothesis of the study of a positive association of HA-related exposure factors and increased PC risk in African-American men. Interestingly, this trend was evident only in the group with self-reported family (brother or father) history of PC and not those without such history. This link between elevated PC risk, family history and a dietary factor supports a role for a polymorphism among the HA metabolism genes (SULT1A, NAT-2, UGT1 and others) that have been linked to HA intake and cancer in other studies. No genotyping was conducted in this study however the use of the archived blood samples for genotyping studies of PC risk in future proposals has been proposed (See Appendix).

3.D. We will also, as feasible, test the validity of Study Hypothesis 2 using combine DOD-funded data set (n = 310) with NIH-only-funded data set (n = 392), during Months 27-36. **Completed as of September 1, 2007. See study abstracts attached. Results of this analysis are presented in Table 1 and indicate that a positive association (OR = 10) between PC risk and elevated HA intake was observed in the combined NIH/DOD data set.**

Table 1: Association of PhIP intake with elevated Prostate-Specific Antigen (PSA) ≥20 ng/mL among 702 African American men, Oakland, CA.

Adjustment or stratification PhIP intake[a] (ng kg^{-1} d^{-1})	PSA ≥20 ng/mL		Odd Ratio (OR)			p_{trend}
			MLE	95% CI[c]		
	m	*n*		Lower	Upper	
<u>All data (%)</u>						
0-50: 6.0	1	355	1	–	–	
>50-75: 19.5	0	176	0.7	0.03	11	
>75-100: 56.2	**5**	**171**	**10.2**	**1.12**	**480**	**0.002**
	PSA ≥20 ng/mL & %fPSA ≤ 25% or Suspicious DRE					
<u>All data (%)</u>						
0-50: 6.0	5	345	1	–	–	
>50-75: 19.5	9	167	**3.7**	**1.1**	**14**	
>75-100: 56.2	16	160	**6.9**	**2.4**	**24**	**0.00005**
<u>FH –</u>						
0-50: 6.0	3	302	1	–	–	–
>50-75: 19.5	9	143	**6.3**	**1.5**	**37**	
>75-100: 56.2	14	134	**10.**	**2.9**	**58**	**0.00004**
<u>FH +</u>						
0-50: 6.0	2	45	1	–	–	
>50-75: 19.5	0	24	0.4	0.01	4.5	
>75-100: 56.2	2	26	1.8	0.12	26	0.44
<u>Adj. for SatFat</u>						
0-50: 6.0	5	345	1	–	–	
>50-75: 19.5	9	167	3.7	1.1	14	
>75-100: 56.2	16	160	6.9	2.4	24	0.0001
<u>Adj. for KCAL</u>						
0-50: 6.0	5	345	1	–	–	
>50-75: 19.5	9	167	3.7	1.1	14	
>75-100: 56.2	16	160	6.9	2.4	24	0.00007

Reportable Outcomes

Abstracts/Posters

Bogen, KT, W Baker, J Chan, D Nelson, E Holly, G Keating, L Paine, and J Felton. 2005. Prostate cancer screening and dietary HA exposure in African-Americans. UCRL-POST-211476. Poster presented at the University of California Davis Health System Future Fair, May 5, 2005, UC Davis Medical Center, Sacramento, CA.

Bogen, KT, GA Keating, EA Holly, J Chan, L Paine, EL Simms, DO Nelson, and J Felton. 2005. Prostate Serum Antigen levels and dietary heterocyclic amines in African Americans: A prospective clinic-based study. Abstract of poster accepted for presentation at the 97th Annual Meeting of the American Assoc. for Cancer Research, April 1-5, 2006, Washington, DC. UCRL-ABS-217085. Lawrence Livermore National Laboratory, Livermore, CA.

Bogen, KT, J Chan, GA Keating, LJ Paine, EL Simms, EA Holly, and JS Felton. 2006. Prostate-specific antigen levels and dietary PhIP in African Americans: A prospective clinic-based study. [Abstract] 2007 Annual Meeting of the American Association for Cancer Research, 14-18 April 2007, Los Angeles, CA. [UCRL-ABS-226551] [see **Appendix** of this report]

Keating, GA, KT Bogen, J Chan, LJ Paine, EL Simms EA Holly and J Felton. 2007. Elevated Prostate-Specific Antigen in African American Men with High Meat-Carcinogen Intake: a Prospective Clinic-Based Study [Abstract] Poster presentation at the DOD Congressionally Mandated Medical Research Program meeting, Innovative Minds in Prostate Cancer Today, September 5-8, 2007, Atlanta, GA. [see **Appendix** of this report]

Reports and other Publications

Bogen KT. 2006. PSA-Based Screening Outcomes, Dietary Heterocyclic Amine Exposure, and Prostate Cancer Risk in African Americans: Annual Report (Year 1 of 3). UCRL-TR-218258. Lawrence Livermore National Laboratory, Livermore, CA.

Keating GA, K Bogen, and J Chan. 2007. Development of a meat frequency questionnaire for use in diet and cancer studies. *J. Am. Dietetic Assoc*. 107, 1356-62. [see **Appendix** of this report]

Bogen KT, GA Keating II, JM Chan, LJ Paine, EL Simms, DO Nelson, and EA Holly. 2007. Highly elevated PSA and dietary PhIP intake in a prospective clinic-based study among African Americans. Prostate Cancer Prostatic Dis. 10:261-9 [see **Appendix** of this report]

Proposals

Chan, J, Keating, G and Paine, L. 2008. Diet, Genetics, Education, and Prostate Cancer Screening in an Outreach Clinic for African-American Men. Abstract from grant proposal submitted to the DOD Congressionally Mandated Medical Research Program – Health Disparities. [see **Appendix** of this report]

Conclusions

Prostate cancer (PC) is the second leading cause of male U.S. cancer deaths, with African-Americans having the highest rate of PC mortality worldwide, as well as more abnormal results from screening tests that correlate with current or eventual PC. A 5-year prospective NIH-funded clinic-based study investigated whether prostate-specific antigen (PSA) and digital rectal exam (DRE) screening indicators of PC risk in 702 African-American men 50 to 70 years of age who underwent PC screening in Oakland, CA (East Bay San Francisco area), were associated with estimated dietary exposures to 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), which forms when meat is overcooked. The DOD-funded study expanded that NIH-funded work by adding a new %-free-PSA test for 310 (108 from the NIH-funded study, plus 202 additional) men, results of which will be compared with PSA/ DRE results and PhIP exposures estimated by dietary interviews. For 392 men studied under the NIH protocol, an odds ratio (95% CL) of 32 (3.2, 720) for highly elevated PSA (≥20 ng/mL) was observed in the highest 15% vs. the lower 50% of estimated daily PhIP intakes. For the final analysis of all 702 men, the corresponding OR was found to be 10 (2.9, 58). This study will help define the potential value of improved screening and dietary/behavioral intervention to reduce PC risk. The dietary/behavioral factors that lead to high levels of PhIP in cooked meats (preference for well done meat and grilled meats) are easily modified through education and intervention. The study has collected unique dietary information from an underserved group at highest risk of PC and will provide valuable insight to target education and intervention programs to this group to reduce PC risk.

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Appendix

Data Table 2: Combined (NIH/DOD) Enrollment, Clinical Findings and Follow-up Results for Project Duration

Data Table 3: Combined (NIH/DOD) Clinical Results and Family PC History for Subjects

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Table 2: Combined (NIH/DOD) Enrollment, Clinical Findings and Follow-up Results for Project Duration

Screen Date	N Scheduled	N_s Screened (enrolled)	Abnormal PSA*	Abnormal DRE*	N_f Followup	N_c (of N_f)[a] Completed	N[a] with-drawn
9/18-19/02	36	29	1	2	3	All, 1L	0
10/31/02	18	13	2	0	2	All 1R	0
11/19/02	18	15	2	1	2	All 1C	0
12/20/02	21	15	1	1	1	All	0
1/14/03	18	18	0	0	0	All	0
2/13/03	21	16	1	0	1	All 1L	1[b]
3/21/03	21	18	2	1	2	All 1C 1W 1N	0
4/10/03	20	13	0	0	0	All	0
5/22/03	20	20	1	0	1	All 1JL	0
6/26/03	19	14	1	1	1	All	0
7/24/03	15	13	0	2	2	All 2L	0
9/17-18/03	28	21	2	2	2	All	0
10/16/03	12	10	0	0	0	All	0
11/1103	21	17	0	0	0	All	0
12/12/03	17	15	0	0	0	All	0
1/22/04	19	16	1	`2	2	ALL,1C 1WL	0
2/6/04	12	11	1	2	3	2LR 1 N	0
3/23/04	13	12	0	1	1	1C	0
4/22/04	17	14	1	1	1	1L	0
5/18/04	18	15	2	0	2	1W, 1C	0
6/8/04	21	15	1	0	1	1 W	0
7/8/04	20	12	1	0	1	1L	0
8/16/04	16	13	0	0	0	0	0
9/21-22/04	33	26	2	2	4	2N, 2RL,	0
11/11/04	16	15	0	1	1	1RL	0
6/23/05	19	18	0	2	2	2RL	0
7/14/05	20	14	0	0	0	all	0
8/25/05	23	17	0	0	0	all	0
9/29/05	18	14	0	1	1	1RL	0
10/26/05	13	10	0	0	0	all	0
12/8/05	17	11	0	0	0	all	0
1/12/06	12	8	1	0	1	W	0
2/8/06	14	9	0	0	0	all	0

3/22/06	22	14	0	0	0	All	0
4/27/06	26	18	2	2	3	1N 2WL	0
5/25/06	20	14	0	0	0	all	0
6/22/06	16	13	2	1	3	2WL1N	0
7/27/06	19	12	1	0	1	1C	
9/14/06	18	12	1	0	1	1W	0
10/12/06	21	10	0	0	0	All	0
11/9/06	13	9	0	0	0	All	0
12/07/06	22	13	0	0	0	All	0
1/4/07	22	10	0	1	1	1N	0
1/25/07	19	13	1	1	2	1RFU, 1W	0.
2/15/07	18	9	1	1	2	1RFU, 1N	0
3/8/07	14	6	0	0	0	all	0
3/29/07	14	9	0	0	0	all	0
4/17/07	13	10	1	0	0	1N	0
5/10/07	24	19	4	0	4	All	0
5/29/07	24	22	2	0	all	2N	0
total	1060	707					

Footnotes to Summary Table

[a]Abnormal PSA = PSA > 4.0 ng/mL. For the purpose of this table, Abnormal DRE totals reflect only abnormal suspicious findings resulting in MCEPC referral for f/u (timely medical follow-up); all other unusual findings (e.g., of BPH) resulted in screenee being advised in writing by MCEPC to discuss results with physician. Total number completed & no longer in f/u = $T_c = N_s - (N_f - N_c)$. A value of N_c = All means that $N_f - N_c = 0$. Follow-up codes are:

R = refused f/u (RFU),

C = cancer diag. in treatment (CIT),

J = lost to f/u due to incarceration (referral to prison clinic),

W = referral made, appt requested at HGH & awaiting appointment or referred to private physician,healthcare facility(VA, Kaiser) being handled by private MD however no follow-up received from MD,

L = Lost to follow-up, no response to multiple phone inquiries, no forwarding phone number and/or address or non compliant keeping appointments

N= normal follow-up results.

Horizontal subdivisions in table refer to SABMC IRB approval/renewal periods.

Table 3: Combined (NIH/DOD) Clinical Results and Family PC History for Subjects

ID	BPH	Urinary	refer	Sus	PSA	%fPSA	Pcfob	ID	BPH	Urinary	refer	Sus	PSA	%fPSA	Pcfob
1	N	N	N	N	2.4		0	46	Y	Y	Y	N	1.2		1
2	Y	N	N	N	2.2		0	47	N	N	N	N	3		0
3	N	N	N	N	0.7		0	48	Y	Y	Y	N	1.2		0
4	N	N	N	N	0.3		0	49	N	N	N	N	0.4		0
5	N	N	N	N	0.8		0	50	N	N	N	N	0.9		0
6	Y	N	N	N	0.6		0	51	N	N	N	N	0.6		0
7	N	N	Y	Y	0.7		0	52	N	N	N	N	1.9		0
8	N	N	N	N	1		0	53	N	N	N	N	0.8		0
9	N	N	N	N	1.1		0	54	N	N	Y	N	33.6		1
10	Y	N	N	N	0.5		0	55	Y	N	N	N	0.4		0
11	N	N	N	N	0.2		0	56	N	N	Y	N	28.6		0
12	Y	N	N	N	2.1		0	57	N	N	N	N	0.9		0
13	N	N	N	N	0.3		0	58	N	N	N	N	0.9		0
14	Y	N	N	N	0.7		0	59	N	N	N	N	0.5		0
15	N	N	N	N	0.3		0	60	N	N	N	N	1.4		0
16	N	N	N	N	0.8		0	61	Y	N	N	N	0.3		1
17	N	N	N	N	0.9		0	62	N	N	N	N	0.3		0
18	N	N	N	N	0.4		0	63	N	N	N	N	0.5		0
19	N	N	N	N	1		0	64	N	N	N	N	0.4		0
20	N	N	N	N	0.8		0	65	N	N	N	N	0.2		0
21	-	N	N	N	1.4		0	66	N	N	Y	N	5.5		1
22	N	N	N	N	2.1		0	67	N	Y	Y	N	0.9		0
23	N	N	N	N	0.4		1	68	N	N	N	N	0.3		0
24	N	N	N	N	4.2		0	69	N	Y	Y	N	0.2		0
25	N	N	N	N	1.1		0	70	N	N	N	N	0.4		0
26	N	N	N	N	0.8		0	71	Y	Y	Y	N	0.1		0
27	Y	N	N	N	2.1		0	72	Y	N	N	N	1.4		0
28	Y	N	N	N	1		0	73	N	N	N	N	0.8		0
29	N	N	N	N	0.6		0	74	N	N	N	N	0.5		0
30	Y	N	N	N	1.3		1	75	N	N	N	N	0.8		0
31	N	Y	Y	N	0.4		1	76	N	N	N	N	1.3		0
32	N	N	N	N	0.5		0	77	Y	N	N	N	0.7		0
33	Y	Y	Y	N	0.6		1	78	N	N	N	N	0.2		0
34	Y	N	Y	N	72.9		0	79	Y	N	N	N	1.3		0
35	N	N	N	N	1.1		1	80	Y	N	N	N	0.9		0
36	Y	N	N	N	1		0	81	Y	N	N	N	1.1		0
37	N	N	N	N	2		0	82	Y	Y	Y	N	2.4		0
38	N	N	N	N	0.2		0	83	Y	Y	Y	N	1.4		1
39	N	N	N	N	4.2		0	84	Y	Y	Y	N	0.5		0
40	N	N	N	N	0.3		0	85	Y	N	N	N	1.4		0
41	N	N	N	N	0.5		0	86	N	N	N	N	0.7		0
42	Y	N	N	N	0.7		0	87	Y	Y	Y	N	0.5		0
43	N	N	N	N	0.9		0	88	Y	N	N	N	0.1		0
44	N	N	N	N	1.6		1	89	Y	Y	Y	N	0.9		0
45	N	N	N	N	1.2		0	90	N	N	N	N	1		0

91	N	N	N	N	0.9	0	136	N	N	N	N	0.3	1
92	N	N	N	N	0.6	0	137	N	Y	Y	N	0.4	0
93	N	N	N	N	0.5	0	138	N	N	N	N	1.5	0
94	N	N	N	N	0.6	0	139	N	N	N	N	0.7	0
95	Y	N	N	N	0.6	0	140	N	N	N	N	1	1
96	N	N	N	N	0.3	0	141	N	N	N	N	3	0
97	N	N	N	N	1	0	142	Y	N	N	N	2.9	0
98	N	N	N	N	0.5	0	143	N	N	N	N	0.6	0
99	N	N	Y	N	54.1	1	144	N	N	Y	N	15.5	0
100	N	N	N	N	0.1	0	145	N	N	N	N	0.3	1
101	N	N	N	N	0.6	0	146	Y	N	N	N	0.05	0
102	N	N	N	N	0.4	0	147	N	N	N	N	1.2	0
103	N	N	N	N	1.3	0	148	Y	N	N	N	0.4	0
104	N	N	N	N	1.5	0	149	N	N	N	N	0.3	0
105	N	N	N	N	0.4	0	150	Y	N	N	N	1	0
106	N	N	N	N	0.4	0	151	N	N	N	N	0.3	0
107	N	N	N	N	0.8	0	152	N	N	N	N	0.05	0
108	N	N	N	N	0.6	0	153	N	N	N	N	2.3	0
109	N	N	N	N	1.6	0	154	Y	N	N	N	1.9	0
110	N	N	N	N	0.4	0	155	N	N	N	N	0.9	0
111	N	N	N	N	1	0	156	N	N	N	N	0.1	0
112	N	Y	Y	N	0.7	0	157	N	N	N	N	0.4	0
113	N	N	N	N	0.7	0	158	N	N	N	N	3.2	0
114	N	N	Y	N	4.6	0	159	Y	Y	Y	N	16.4	0
115	N	N	N	N	0.1	0	160	N	N	N	N	0.4	0
116	N	N	Y	Y	1.3	0	161	N	N	N	N	1.4	0
117	N	N	N	N	0.7	0	162	N	Y	Y	N	0.4	0
118	N	N	Y	N	12.8	0	163	N	N	N	N	1.7	0
119	N	N	N	N	0.5	0	164	Y	Y	Y	N	0.5	0
120	N	N	N	N	0.7	0	165	N	N	N	N	0.3	0
121	N	N	N	N	1.2	0	166	N	N	N	N	1.1	0
122	Y	N	N	N	0.5	0	167	N	Y	Y	N	1.1	0
123	Y	N	N	N	0.7	0	168	N	N	N	N	1.4	0
124	Y	N	N	N	2.9	0	169	N	N	N	N	0.8	0
125	N	N	N	N	0.3	0	170	N	N	N	N	0.9	0
126	N	N	N	N	0.4	0	171	N	Y	Y	N	3	0
127	N	N	N	N	1	0	172	N	N	N	N	0.3	0
128	N	N	N	N	0.8	1	173	N	N	N	N	0.5	0
129	N	Y	Y	N	1	0	174	N	N	Y	Y	3.5	0
130	N	N	N	N	0.8	0	175	N	N	N	N	0.9	0
131	N	N	N	N	0.6	0	176	Y	N	N	N	0.9	0
132	Y	N	N	N	1.7	0	177	N	N	N	N	0.4	0
133	N	N	N	N	0.2	1	178	N	N	N	N	1.5	0
134	N	N	N	N	1.4	0	179	N	N	N	N	0.5	0
135	N	N	N	N	3.2	0	180	N	N	Y	Y	0.3	0

181	N	N	N	N	0.5	0	226	Y	Y	Y	N	0.6	0
182	N	N	N	N	2.4	0	227	N	N	N	N	0.3	1
183	N	N	N	N	1.9	0	228	N	N	N	N	1.7	0
184	N	N	N	N	0.5	0	229	Y	Y	Y	N	1.3	0
185	N	N	Y	N	8.3	0	230	Y	N	N	N	0.1	1
186	N	N	N	N	1.9	0	231	N	N	N	N	0.8	0
187	N	N	N	N	1.8	0	232	N	N	N	N	0.5	0
188	N	N	N	N	1.7	0	233	N	N	N	N	1.1	0
189	Y	Y	Y	N	10.2	0	234	N	N	N	N	0.5	0
190	N	N	N	N	0.8	0	235	N	N	N	N	0.4	0
191	N	N	Y	N	0.1	0	236	N	N	N	N	0.6	0
192	Y	N	N	N	1	1	237	N	N	N	N	1	0
193	N	N	N	N	0.7	0	238	Y	Y	Y	N	2	1
194	N	N	N	N	0.6	0	239	N	N	N	N	0.8	0
195	Y	N	N	N	0.4	0	240	N	N	N	N	1.2	0
196	Y	N	N	N	3.3	0	241	N	N	N	N	2.4	0
197	N	N	N	N	0.5	0	242	N	N	N	N	1	0
198	N	N	N	N	0.8	0	243	N	N	N	N	0.5	0
199	N	N	N	N	0.9	0	244	N	N	Y	Y	17.3	0
200	N	N	N	N	2.2	0	245	N	N	N	N	0.4	0
201	N	N	N	N	0.4	0	246	Y	N	N	N	0.8	0
202	Y	N	N	N	1.1	0	247	N	N	N	N	0.4	0
203	N	N	N	N	0.9	0	248	Y	N	N	N	0.6	0
204	N	N	N	N	1.2	0	249	N	Y	N	N	0.2	0
205	N	N	N	N	0.4	0	250	N	N	N	N	0.5	0
206	Y	N	N	N	0.7	0	251	N	N	N	N	0.7	0
207	N	N	N	N	1.3	0	252	N	N	N	N	1.2	0
208	Y	N	N	N	1	0	253	Y	N	N	N	0.5	0
209	N	N	N	N	0.2	0	254	N	n	N	N	1.6	0
210	N	N	N	N	1.3	0	255	N	Y	N	N	1.1	0
211	N	N	N	N	0.7	1	256	Y	Y	N	N	1.1	0
212	N	N	N	N	1	0	257	Y	Y	N	N	1.1	0
213	N	N	N	N	1.3	0	258	N	Y	N	N	1.2	0
214	N	N	N	N	0.8	0	259	N	N	Y	Y	0.6	0
215	N	N	N	N	0.5	0	260	Y	Y	N	N	3.1	0
216	N	N	N	N	1.6	0	261	N	N	Y	Y	0.7	1
217	N	Y	Y	N	0.9	1	262	N	N	N	N	0.1	0
218	N	N	N	N	1.5	0	263	N	N	N	N	0.3	0
219	Y	N	N	N	0.7	0	264	N	N	N	N	0.2	0
220	Y	N	N	N	2.3	0	265	Y	N	N	N	1.3	0
221	Y	Y	Y	N	0.7	0	266	N	N	N	N	2.7	0
222	N	Y	Y	N	0.2	0	267	N	N	Y	Y	1.2	0
223	N	N	N	N	1.4	0	268	Y	N	Y	N	11.6	0
224	N	N	N	N	3.5	0	269	N	N	N	N	1.4	0
225	N	Y	Y	N	0.4	0	270	N	N	N	N	1.6	0

271	N	N	N	N	0.9	0	316	N	N	Y	N	8.4	0
272	Y	Y	N	N	1.3	1	317	Y	N	N	N	0.4	1
273	N	N	N	N	1.5	0	318	N	N	N	N	0.5	0
274	Y	Y	N	N	1	0	319	N	N	N	N	0.9	0
275	Y	N	N	N	2.6	0	320	N	N	N	N	1.3	0
276	N	N	N	N	3.7	0	321	N	N	N	N	0.3	0
277	Y	N	N	N	1.3	0	322	N	N	N	N	0.3	0
278	Y	N	N	N	1.1	0	323	Y	N	N	N	0.6	1
279	Y	Y	N	N	1.2	1	324	N	Y	N	N	1	1
280	Y	Y	N	N	0.7	0	325	Y	N	N	N	0.5	1
281	Y	N	N	N	2.3	0	326	N	N	N	N	0.3	0
282	N	N	Y	Y	2.1	0	327	N	N	N	N	0.4	0
283	N	N	N	N	1.6	0	328	Y	N	N	N	2.3	0
284	N	N	N	N	1.2	0	329	Y	N	N	N	2.3	0
285	N	N	N	N	0.9	0	330	Y	N	N	N	0.7	0
286	N	N	Y	Y	4.4	0	331	Y	N	N	N	1.1	0
287	Y	N	N	N	1	1	332	N	N	N	N	0.8	0
288	N	N	N	N	0.7	0	333	N	N	N	N	1.3	0
289	N	N	N	N	0.4	0	334	Y	N	N	N	0.9	0
290	N	N	N	N	1.5	0	335	N	N	N	N	0.5	0
291	N	N	N	N	0.3	0	336	N	N	N	N	0.9	0
292	N	N	N	N	0.7	0	337	N	N	N	N	0.9	0
293	N	N	N	N	0.1	0	338	N	N	Y	N	5.9	0
294	N	N	N	N	0.4	0	339	Y	N	N	N	1	1
295	N	N	N	N	0.9	0	340	Y	N	N	N	0.9	0
296	N	N	N	N	0.7	1	341	N	N	N	N	1.6	0
297	N	N	Y	N	48.1	0	342	Y	Y	N	N	2.8	0
298	Y	N	N	N	0.5	0	343	Y	N	N	N	1.6	0
299	N	N	N	N	0.6	0	344	N	N	N	N	0.5	0
300	Y	N	N	N	1.5	0	345	N	N	N	N	0.6	0
301	N	N	Y	N	4.9	0	346	Y	N	N	N	0.7	0
302	Y	N	N	N	3	0	347	N	N	N	N	0.9	0
303	N	N	N	N	0.9	0	348	Y	N	N	N	0.7	0
304	N	N	N	N	0.2	0	349	Y	N	N	N	1.3	0
305	Y	N	N	N	3.5	0	350	N	N	N	N	1.3	0
306	Y	N	N	N	0.6	1	351	Y	N	N	N	1.3	0
307	N	N	N	N	0.6	0	352	N	N	N	N	0.8	0
308	Y	N	N	N	3.5	0	353	Y	Y	N	N	1.1	0
309	Y	N	N	N	0.5	1	354	N	N	Y	Y	3.3	0
310	Y	N	N	N	0.9	0	355	Y	N	N	N	3	1
311	Y	N	N	N	0.3	0	356	Y	N	N	N	0.6	0
312	N	N	N	N	0.7	1	357	N	N	Y	N	4.7	0
313	Y	Y	N	N	1.5	0	358	N	N	N	N	2.4	0
314	Y	Y	N	N	1	0	359	Y	Y	N	N	1.3	0
315	N	N	N	N	1.1	0	360	N	N	N	N	0.9	0

361	Y	N	N	N	0.3		1	406	N	N	N	N	0.5	0.05	0
362	N	N	N	N	3.1		1	407	N	N	N	N	0.4	0.15	0
363	N	N	N	N	1.1		0	408	Y	N	N	N	3.9	1.78	0
364	N	N	N	N	0.8		0	409	N	N	N	N	3.7	0.31	0
365	N	N	N	N	0.9		0	410	N	N	N	N	0.2	0.05	0
366	N	N	N	N	2.4		1	411	N	N	N	N	1.4	0.38	0
367	Y	N	N	N	0.8		0	412	Y	N	N	N	1.7	0.35	0
368	Y	Y	N	N	1.3		1	413	N	N	N	N	0.5	0.1	0
369	N	N	Y	Y	14.6		0	414	Y	N	N	N	0.8	0.2	1
370	Y	N	N	N	2.4		0	415	Y	Y	N	N	1.7	0.28	0
371	N	N	N	N	0.6		0	416	N	N	N	N	0.6	0.12	1
372	N	N	N	N	0.6		0	417	N	N	N	N	0.3	0.16	0
373	N	N	Y	Y	0.7		0	418	Y	N	N	N	2.7	0.33	0
374	N	N	N	N	0.5		1	419	N	N	N	N	0.8	0.3	1
375	N	N	N	N	0.5		0	420	Y	N	N	N	3.8	0.47	1
376	N	N	N	N	1.2		0	421	N	N	N	N	1	0.32	1
377	N	N	N	N	0.4		0	422	N	N	N	N	0.1	0.05	0
378	Y	N	N	N	0.4		0	423	N	N	N	N	0.8	0.22	0
379	N	N	N	N	0.7		0	424	Y	Y	N	N	1.1	0.31	0
380	N	N	N	N	0.6		0	425	Y	N	N	N	0.7	0.12	1
381	N	N	N	N	0.3		0	426	N	N	N	N	1.1	0.15	1
382	N	N	N	N	0.6		0	427	Y	Y	N	N	0.7	0.18	0
383	Y	Y	N	N	1.6		0	428	N	N	N	N	0.6	0.22	0
384	N	Y	Y	Y	3.3		0	429	N	N	N	N	0.3	0/a	0
385	N	N	N	N	0.5		0	430	N	N	N	N	1.3	0.32	0
386	N	N	N	N	0.1		0	431	N	Y	N	N	1.6	0.25	0
387	N	N	N	N	1.5		0	432	Y	N	N	N	0.8	0.34	1
388	N	N	N	N	0.4		1	433	N	N	N	N	1.2	0.21	0
389	Y	N	N	N	0.5	0.21	0	434	Y	N	N	N	0.9	0.14	1
390	Blank	Y	Blank	Y	0.9	Blank	0	435	N	Y	N	N	0.4	0.15	0
391	N	N	N	N	0.5	0.14	0	436	N	N	N	N	0.4	0.11	0
392	N	N	N	N	1.9	0.52	0	437	Y	Y	N	N	4.7	1.72	0
393	N	N	N	N	0.4	0.05	0	438	N	N	N	N	0.6	0.15	0
394	N	N	N	N	1	0.15	0	439	N	N	N	N	1.1	0.15	0
395	N	N	N	N	0.4	0.12	0	440	N	N	N	N	0.4	0.11	0
396	N	N	N	N	0.8	0.29	1	441	N	N	N	N	0.2	0.05	0
397	Y	N	N	N	0.3	0.16	0	442	N	N	N	N	0.8	0.4	0
398	N	N	Y	Y	3.7	0.57	0	443	N	N	N	N	0.9	0.33	0
399	N	N	N	N	0.5	0.25	0	444	N	N	N	N	0.1	0.05	1
400	N	N	Y	Y	3.4	0.36	0	445	N	N	N	N	0.6	0.19	1
401	N	N	N	N	1.3	0.24	1	446	N	N	N	N	0.3	0.05	0
402	N	N	N	N	0.9	0.41	1	447	N	N	N	N	0.3	0.14	0
403	N	N	N	N	0.7	0.39	1	448	N	N	N	N	1.4	0.21	0
404	N	N	N	N	1.2	0.39	1	449	N	N	Y	Y	1.7	0.22	0
405	Y	N	N	N	2.2	0.32	0	450	N	N	N	N	0.3	0.13	0

451	N	N	N	N	0.7	0.26	0	496	N	N	N	N	0.7	0.12	0
452	N	N	N	N	0.4	0.05	0	497	N	N	N	N	0.3	0.05	0
453	N	N	N	N	1.2	0.29	0	498	N	N	N	N	0.4	0.13	0
454	N	N	N	N	0.5	0.11	0	499	N	N	N	N	0.3	0.18	0
455	N	Y	N	N	0.8	0.13	0	500	N	N	N	N	1.6	0.34	0
456	N	N	Y	Y	2.6	1.16	1	501	N	N	N	N	0.1	0.05	1
457	N	N	N	N	4.2	1.4	1	502	N	N	N	N	0.3	0.05	0
458	Y	N	N	N	1	0.37	0	503	N	N	N	N	2	0.2	0
459	N	N	N	N	0.5	0.25	0	504	Y	Y	N	N	2.3	0.39	0
460	N	N	N	N	0.8	0.2	0	505	N	N	N	N	1.3	0.39	0
461	N	N	N	N	0.1	0.05	0	506	Y	N	N	N	1	0.19	0
462	Y	N	N	N	1.6	0.31	0	507	Y	N	N	N	0.6	0.22	1
463	N	N	N	N	0.3	0.16	0	508	Y	N	N	N	3.2	0.79	0
464	Y	Y	N	N	0.8	0.31	0	509	Y	N	N	N	0.2	0.05	0
465	N	N	N	N	0.3	0.11	0	510	N	N	N	N	0.7	0.19	0
466	Y	N	N	N	1.6	0.33	0	511	Y	N	N	N	0.4	0.11	0
467	N	N	N	N	3.6	0.55	0	512	N	N	N	N	2.3	0.36	0
468	Y	N	N	N	2.4	0.7	0	513	N	N	Y	Y	0.5	0.16	0
469	Y	Y	N	N	0.6	0.19	0	514	Y	Y	N	N	0.3	0.05	0
470	N	N	N	N	0.1	0.05	0	515	Y	N	N	N	1.7	0.25	0
471	Y	N	N	N	1.9	0.29	0	516	Y	Y	N	N	0.7	0.25	0
472	Y	N	N	N	1.7	0.38	0	517	N	N	Y	Y	10.6	2.64	0
473	Y	N	N	N	2	0.36	1	518	Y	N	N	N	5.8	0.56	0
474	N	N	N	N	0.6	0.27	0	519	N	Y	N	N	0.5	0.05	1
475	N	N	N	N	0.6	0.11	1	520	Y	Y	N	N	1.5	0.31	0
476	Y	N	N	N	0.2	0.05	0	521	N	N	N	N	0.1	0.05	0
477	N	N	Y	N	5.3	1.45	0	522	Y	N	N	N	0.2	0.11	0
478	N	N	N	N	0.2	0.05	0	523	Y	Y	N	N	1.5	0.44	1
479	N	N	N	N	3	0.39	1	524	N	N	N	N	1.6	0.52	0
480	N	N	N	N	2.3	0.3	0	525	N	N	N	N	0.3	0.14	0
481	N	N	N	N	3.3	0.55	0	526	Y	Y	N	N	1.5	0.33	0
482	N	N	N	N	0.8	0.2	0	527	N	N	N	N	0.2	0a	0
483	N	N	N	N	1.2	0.3	0	528	N	N	N	N	0.5	0.15	0
484	N	N	N	N	1.1	0.1	1	529	Y	Y	N	N	0.7	0.43	0
485	N	N	N	N	0.5	0.17	0	530	Y	N	N	N	1.4	0.37	0
486	Y	N	N	N	1.2	0.11	0	531	N	Y	N	N	1.4	0.38	1
487	N	N	N	N	0.3	0.19	0	532	Y	Y	N	N	2.4	0.68	1
488	N	Y	N	N	2.3	0.28	0	533	Y	N	N	N	0.6	0.33	0
489	Y	Y	N	N	3.7	0.44	0	534	Y	N	N	N	0.5	0.19	0
490	N	Y	N	N	3.1	0.25	0	535	N	N	N	N	0.7	0.18	0
491	N	N	N	N	1.4	0.22	0	536	N	N	N	N	0.9	0.14	0
492	N	N	N	N	0.8	0.37	0	537	N	Y	N	N	0.7	0.16	0
493	N	N	N	N	0.8	0.12	0	538	N	N	N	N	0.7	0.23	0
494	N	N	N	N	0.5	0.05	0	539	N	N	N	N	0.5	0.13	0
495	N	Y	N	N	0.8	0.14	0	540	N	N	Y	Y	1.6	0.3	0

541	Y	N	Y	N	9.5	0.94	1	586	N	N	N	N	0.6	0.25	0
542	N	N	N	N	2	0.26	1	587	Y	Y	N	N	0.2	0.18	1
543	Y	N	N	N	0.2	0.05	0	588	N	N	N	N	1.3	0.22	0
544	N	N	Y	N	5	0.38	1	589	Y	N	N	N	1.4	0.78	0
545	Y	N	N	N	0.4	0.11	1	590	N	N	N	N	0.3	0.13	0
546	Y	N	N	N	0.2	0.13	0	591	N	N	N	N	0.05	0.05	1
547	N	N	N	N	1.3	0.33	1	592	N	N	N	N	0.5	0.14	0
548	N	N	N	N	0.9	0.25	0	593	N	Y	N	N	0.5	0.05	1
549	N	N	N	N	0.2	0.05	0	594	N	N	N	N	1.7	0.36	0
550	N	N	N	N	1	0.24	0	595	N	N	N	N	1.1	0.22	0
551	N	N	N	N	0.7	0.21	1	596	N	Y	Y	N	2.9	1.27	0
552	Y	N	N	N	0.3	0.1	0	597	N	N	N	N	1.2	0.27	0
553	N	N	N	N	2.1	0.41	0	598	N	N	N	N	1.6	0.48	0
554	N	N	N	N	9.3	0.61	0	599	N	N	N	N	1.5	0.37	1
555	N	N	N	N	0.7	0.05	0	600	N	N	N	N	0.4	0.13	0
556	N	N	N	N	1.3	1.3	0	601	N	N	N	N	1.4	0.30	0
557	Y	N	N	N	1.1	0.38	0	602	N	N	N	N	0.5	0.15	1
558	Y	N	N	N	0.3	0.05	0	603	Y	N	N	N	1.9	0.31	0
559	N	N	N	N	0.7	0.2	0	604	N	N	N	N	0.4	0.14	0
560	N	N	N	N	0.3	0.16	0	605	N	N	N	N	0.7	0.05	2
561	N	N	N	N	0.1	0.05	0	606	N	N	Y	Y	2.4	0.43	0
562	N	N	N	N	0.1	0.05	0	607	Y	N	N	N	0.7	0.17	0
563	N	N	N	N	0.3	0.18	0	608	N	N	N	N	1.2	0.10	1
564	N	N	N	N	0.7	0.28	1	609	N	N	N	N	2.6	0.60	0
565	N	N	N	N	0.6	0.13	0	610	N	N	N	N	0.8	0.16	0
566	N	N	N	N	1	0.21	0	611	Y	N	N	N	1.7	0.30	0
567	N	N	N	N	0.5	0.23	0	612	Y	N	N	N	1.8	0.38	0
568	Y	N	N	N	0.2	0.05	0	613	N	N	N	N	0.2	0.05	0
569	Y	Y	N	N	13.4	1.06	0	614	Y	Y	N	N	2.6	0.89	0
570	Y	N	N	N	0.9	0.20	0	615	N	N	N	N	0.6	0.35	0
571	N	N	N	N	0.6	0.12	0	616	Y	N	N	N	0.2	0.05	0
572	N	N	N	N	0.4	0.16	0	617	N	N	N	N	0.6	0.16	0
573	Y	N	N	N	0.3	0.05	0	618	N	N	N	N	1.3	0.23	0
574	N	N	N	N	0.6	0.21	0	619	N	N	N	N	7.1	0.81	0
575	N	N	N	N	0.3	0.11	1	620	N	N	N	N	0.6	0.16	0
576	Y	N	N	N	0.4	0.05	0	621	Y	N	N	N	1.7	0.53	0
577	N	N	N	N	0.5	0.16	0	622	Y	N	N	N	1.2	0.43	0
578	Y	N	N	N	0.3	0.10	0	623	N	N	N	N	1.4	0.32	0
579	N	N	N	N	0.5	0.05	0	624	Y	N	N	N	0.5	0.23	0
580	Y	N	N	N	0.6	0.11	0	625	N	N	Y	Y	1.2	0.20	0
581	Y	Y	N	N	1	0.18	0	626	N	N	N	N	0.6	0.19	0
582	Y	N	N	N	0.5	0.21	1	627	Y	N	N	N	2.4	0.64	0
583	Y	N	N	N	0.7	0.14	0	628	Y	N	N	N	3	0.36	0
584	Y	N	N	N	2.7	0.79	0	629	N	N	N	N	0.6	0.25	0
585	Y	N	N	N	0.4	0.20	0	630	N	N	N	N	0.4	0.12	0

631	Y	N	N	N	1.1	0.20	0	671	Y	Y	N	N	0.5	0.18	0
632	N	N	N	N	0.9	0.18	0	672	Y	N	N	N	0.3	0.13	0
633	N	N	N	Y	0.6	0.13	0	673	N	N	N	N	0.9	0.33	1
634	N	N	N	N	0.4	0.12	0	674	Y	Y	N	N	6.3	0.62	0
635	Y	N	N	N	7.1	0.91	1	675	Y	Y	N	N	1.5	0.32	0
636	N	N	N	N	0.4	0.21	0	676	N	N	N	N	0.7	0.23	1
637	N	N	N	N	1	0.22	1	677	Y	N	N	N	0.6	0.16	0
638	Y	Y	N	N	0.6	0.14	0	678	N	N	N	N	0.3	0.05	0
639	Y	Y	N	N	0.5	0.25	0	679	N	N	N	N	0.2	0.05	0
640	Y	Y	N	N	1	0.26	0	680	N	N	N	N	30.5	4.47	0
641	Y	Y	N	N	0.8	0.25	0	681	Y	N	N	N	0.6	0.18	0
642	Y	Y	N	N	0.7	0.05	0	682	Y	N	N	N	5.8	0.50	0
643	Y	Y	N	N	2.4	0.43	0	683	N	N	N	N	1.6	0.47	0
644	N	Y	N	N	0.7	0.28	0	684	Y	Y	N	N	1.7	0.23	0
645	N	N	N	N	0.3	0.15	0	685	N	N	N	N	7.7	0.73	1
646	Y	Y	N	N	0.6	0.30	0	686	Y	Y	N	N	17.4	2.03	0
647	Y	N	N	N	0.6	0.05	0	687	N	N	N	N	0.5	0.12	0
648	N	N	N	N	0.7	0.24	0	688	N	N	N	N	0.3	0.14	0
649	Y	N	N	N	1.9	0.52	0	689	Y	N	N	N	0.6	0.20	0
650	Y	N	N	N	0.2	0.05	0	690	Y	Y	N	N	0.4	0.13	0
651	Y	Y	N	N	1.2	0.44	0	691	N	N	N	N	0.3	0.11	1
652	Y	Y	N	N	1.2	0.34	0	692	Y	N	N	N	6.5	1.69	0
653	Y	N	N	N	0.5	0.11	0	693	Y	Y	N	N	1.9	0.19	0
654	Y	Y	N	N	0.9	0.37	0	694	N	N	N	N	0.8	0.17	0
655	N	N	N	N	0.9	0.26	0	695	N	N	N	N	2.4	0.43	0
656	Y	N	N	N	0.5	0.16	0	696	Y	Y	N	N	0.4	0.25	0
657	N	Y	Y	N	1.5	0.20	0	697	Y	N	N	N	0.3	0.17	0
658	Y	N	N	N	0.3	0.10	2	698	N	N	N	N	0.3	0.05	0
659	Y	N	N	N	4.7	0.77	0	699	N	N	N	N	0.6	0.22	1
660	Y	N	N	N	0.6	0.22	0	700	N	N	N	N	1.1	0.13	0
661	Y	N	N	N	1	0.22	0	701	N	N	N	N	2.2	0.37	0
662	Y	N	N	N	1.2	0.21	0	702	Y	Y	N	N	0.2	0.05	0
663	N	N	N	N	0.6	0.20	1								
664	Y	N	N	N	1.3	0.30	1								
665	Y	N	N	N	3.1	0.59	0								
666	Y	N	N	N	0.9	0.30	0								
667	N	N	N	N	1.5	0.57	0								
668	N	N	N	N	0.8	0.35	0								
669	Y	N	N	N	0.7	0.17	0								
670	Y	N	N	N	1.8	0.33	0								

ID: Subject identifier
BPH: benign prostatic hyperplasia
Urinary: urinary symptoms
Refer: referred to follow-up
Sus: suspicious digital rectal exam
PSA: prostate specific antigen level
%fPSA: percent free prostate specific antigen
Pcfob: prostate cancer diagnosed in father or brother

ORIGINAL ARICLE

Highly elevated PSA and dietary PhIP intake in a prospective clinic-based study among African Americans

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Abbreviations (CAS #): HA = heterocyclic amine, PC = prostate cancer, PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (105650-23-5).

ABSTRACT

African American men die from prostate cancer (PC) nearly twice as often as white U.S. men and consume about twice as much of the predominant U.S. dietary heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), a genotoxic rat-prostate carcinogen found primarily in well-cooked chicken and beef. To investigate the hypothesis that PhIP exposure increases PC risk, an ongoing prospective clinic-based study compared PC-screening outcomes with survey-based estimates of dietary PhIP intake among 40-70-year-old African American men with no prior PC in Oakland, CA. They completed food-frequency and meat-cooking/consumption questionnaires and had a prostate-specific antigen (PSA) test and digital-rectal exam. Results for 392 men indicated a 17 (±17) ng $kg^{-1}d^{-1}$ mean (±1SD) daily intake of PhIP, about twice that of white U.S. men of similar age. PhIP intake was attributable mostly to chicken (61%) and positively associated (R^2=0.32, p<0.0001) with saturated fat intake. An odds ratio (95% CI) of 31 (3.1-690) for highly elevated PSA ≥20 ng/mL was observed in the highest 15% vs. lowest 50% of estimated daily PhIP intake (≥30 vs ≤10 ng $kg^{-1}d^{-1}$) among men 50+ years old (p=0.0002 for trend) and remained significant after adjustment for self-reported family history of (brother or father) PC, saturated fat intake, and total energy intake. PSA measures were higher in African American men with positive family history (p=0.007 all men, p<0.0001 highest PSA quartile). These preliminary results are consistent with a positive association between PhIP intake and highly elevated PSA, supporting the hypothesis that dietary intervention may help reduce PC risk.

Introduction

Heterocyclic amines (HAs) are potent mutagens formed in meats, chicken and fish as it is cooked to higher-doneness levels by heat-intensive cooking methods.[1-3] HAs also cause cancer in a variety of tissue types in multiple bioassays in different animal species, strains and sexes, and so may present a human dietary cancer risk.[4,5] A predominant HA found particularly in well-done chicken and beef is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP).[6-11] Dietary exposure to PhIP has been shown to induce colon, intestinal and mammary adenocarcinomas in rats[12-18] and prostate cancer in rats.[19-20] HAs including PhIP are positive in numerous genotoxicity assays.[21-22] Like other HAs, PhIP is metabolically activated by P450 and *N*-acetyltransferase enzymes to genotoxic metabolites, which in the case of PhIP mutate both rat and human prostate DNA.[21,23-40] PhIP also has potent estrogenic activity that may act synergistically with genotoxic effects to enhance its carcinogenicity.[41]

In the U.S., prostate cancer (PC) is a leading cause of cancer death among men, with African Americans having the highest age-specific prostate cancer rate in the world, and a >2-fold higher rate of mortality for PC than white men in the U.S.[42-44] Although family history of PC (particularly father/brother) is clearly linked to substantially elevated PC risk[45-48] and a human-PC-specific chromosome translocation has been identified[49], there is no evidence for a predominant heritable factor for PC. Racial-ethnic differences in testosterone-related hormone and/or receptor levels may also affect racial-ethnic differences in PC risk, but their role remains unclear.[50-52]

Substantial geographical variations in PC incidence indicate the importance of one or more dietary or other environmental factors.[53-55] Dietary intake of animal or saturated fat often has been linked to increased PC risk in previous studies, including among African American men. However, these associations appear too weak to explain more than a small fraction of observed racial-ethnic differences in PC risk.[56-59] This also appears to be the case for other environmental/dietary factors examined such calcium, cruciferous vegetables, vitamin D, UV from sunlight, lycopene, and body size.[60-66] Because cooked-meat intake is positively associated with total saturated-fat intake[67], previous studies that focused on animal or saturated fat *per se* may have estimated effects due largely or entirely to meat-related HA intake.

Consumption of cooked meats and associated HAs have been linked to increased risks of colorectal adenoma/adenocarcinoma and of cancer of the stomach, breast, lung and prostate[68-90], whereas fewer studies have reported no such associations[89,91-94] One study[94] quantified HA-specific intake estimates (including for PhIP), but detected no positive association of consumption of any HA (from white or red meat) with elevated risk of breast cancer, regardless of *N*-acetyltransferase genotype. Other positive studies have tended to include those with HA exposure quantified after adjusting for factors expected to determine HA intake—namely, meat type, consumption frequency, cooking method, and cooking doneness. For example, recently published results from a study that used

the Block Brief 2000 survey to assess meat-related exposures detected elevated PC risk among 692 African American men (but not among nearly 65,000 white men) who consumed relatively more cooked red or processed meats.[89]

Periodic screening for serum levels of Prostate-Specific Antigen (PSA) is useful to detect early PC and benign prostatic hyperplasia (BPH) because PSA levels in serum are positively associated with age, prostate volume, and prostatic neoplastic disease.[95-97] Significantly higher PSA levels are found in African Americans than in whites, even after adjustment for age and prostate volume in men without PC, and for PC grade and stage in men with PC.[98-100] This is due to greater PSA secretion per unit prostate volume by African American men.[101] Although a substantial fraction of "slightly" elevated PSA levels (between 4 and 10 ng/mL) is attributable to BPH or infection, "highly elevated" PSA levels (≥20 ng/mL) typically indicate a strong likelihood of localized or metastatic PC, with >99% specificity[102-104] and with >95% positive predictivity for men of age 50-69.[105] Likewise, even among men with a PSA measure <4.0 ng/mL (often considered within the "normal" range), it is estimated that about 15% later may be diagnosed with PC.[106]

To investigate further whether PhIP intake is related to PC risk, we conducted a study to assess the association between highly elevated PSA as a screening indicator for elevated PC risk and estimated dietary exposure to PhIP among African American men.

Methods

Study design and participants. In an ongoing clinic-based prospective study using Institutional Review Board-approved human participant protocols, we enrolled 392 African American men from the Oakland, CA area using the following inclusion criteria: (1) African American men between 40 and 70 years old; (2) no previous PC diagnosis or contraindicated medical condition; and (3) written informed consent. Participation was enhanced by a $30 incentive payment and by >10 years of previous PC-related community outreach undertaken by the study clinic (the Markstein Cancer Education and Prevention Center at Alta Bates Summit Medical Center in Oakland, CA). After providing written informed consent, each participant completed a PC-screening medical questionnaire, answered general and detailed meat-related dietary questions, and was provided free PC screening including a PSA blood test and a digital-rectal exam (DRE) by a board-certified urologist.

Oakland is a metropolitan city near San Francisco within California's Alameda County, in which prostate cancer is the most common cancer in African American men with about 920 new cases predicted in 2006.[107] In 2004, Alameda County had a 14.1% African American population, whereas in Oakland, the site of the medical center where our study was conducted, 35.7% of the population was African American, and African American

populations statewide and nationwide in 2004 were 6.8% and 12.8%, respectively.[108] African American men in the Alameda County recruitment area have a median age of 34 years, median per capita income of about $34,700, and a currently-married rate of 39%.[108] Outreach was tailored to African American men ages 40-70 in Alameda County reflecting its current socio-economically diverse African American population, including a large fraction of unmarried men who do not benefit from positive spousal influences on health-care choices. Outreach was conducted at more sites frequented by men including barbershops catering to African Americans, social service organizations, African-American churches, senior centers and housing units, Veterans Administration out-patient clinics, community health clinics and medical practices of members of the Sinkler-Miller Medical Association, a professional association of African American physicians.

Dietary Assessment. General dietary intake over the previous year was estimated using the Block-2000 food-frequency questionnaire (FFQ) with portion-size standardized food-model photographs to help each participant select portion sizes.[109] Dietary data on specific meats consumed and preferred cooking methods over the previous year were obtained using an additional questionnaire that includes a validated set of standard meat-doneness descriptors and corresponding set of meat-doneness photographs.[110-112] All dietary questionnaire data were obtained by in-person interviews administered by trained dietary interviewers.

Data Analysis. Combined survey data were used as previously described[113-115] to estimate annual average dietary PhIP intake from all sources by each participant. Total and basal energy intake in kcal per kg body weight was estimated for each study participant using previously described methods.[113] Standard methods were used to assess the significance of linear associations and outliers where noted, and to assess Pearson product-moment correlations.[116-117] Approximate significance of differences in mean HA-intake was compared using Welch's T-test whereas unequal variance was assessed by corresponding F-tests. Differences in PSA by family history were assessed by the Wilcoxon test[118] Odds ratio (OR) and corresponding 95% confidence interval (CI) estimates obtained by numerical maximum-likelihood procedures are reported together with corresponding chi-square tests for trend (with or without adjustment for specified factors).[119] Reported Fisher exact p-values are for 2-tailed tests. Significance p-values $\leq 10^{-10}$ were reported as being ≈ 0, and values <0.10 were reported to one significant figure. All calculations were done using *Mathematica* 5.2® software.[120]

Results

PhIP intake. Data on 392 African American men who participated in this study are summarized in Table 1. Corresponding estimated average daily intake of specific meats and total PhIP is summarized in Table 2. The empirical distribution of estimated daily intake of PhIP from all meats ("total PhIP") had geometric and arithmetic

mean values of 9.6 and 17 ng kg^{-1} d^{-1}, respectively, and a geometric standard deviation of 3.3. A total of 89% of inter-individual variance in PhIP intake could be explained by intake of specific meats and the cooking method of those meats, and doneness-preference data appeared to explain the remaining 11% of inter-individual variation in PhIP intake. Ratios of total to basal daily intake rates of energy per unit body weight estimated for this study population had an arithmetic mean (±1 standard error of the mean) of 1.57 (±0.046), not significantly different ($p = 0.51$) from the value of 1.6 expected for reference adult men.[113]

Correlation between PhIP and other dietary measures. Estimated daily intake of total PhIP explained approximately 32% of observed inter-individual variance in corresponding estimated intake of saturated fat per unit body weight (Figure 1). Similar or greater levels of positive correlation were observed between estimated total PhIP intake and energy-intake ratio (E_{food}:E_{basal}) ($R^2 = 0.26$), total energy intake (E_{food}) ($R^2 = 0.27$), and total meat intake (g kg^{-1} d^{-1}) ($R^2 = 0.68$). There were strong correlations between estimated intake of total energy and saturated fat ($R^2 = 0.84$) and between total energy and E_{food}:E_{basal} ($R^2 = 0.97$).

PSA, age, and family history. PSA measures were weakly positively associated with participant age and attained statistical significance for all measures <4 ng/mL ($R^2 = 0.051$, $p = 0.00001$), but not for all measures ≥4 ng/mL ($R^2 = 0.035$, $p = 0.41$). PSA measures ≥20 ng/mL were observed only among older participants, aged 55 to 65 years. PSA measures among participants reporting vs. not reporting a father and/or brother with PC were greater among those reporting such a family history ($p = 0.007$ by Wilcoxon test), particularly when the comparison was restricted to the upper quartile of PSA measures in each family-history category ($p = 0.0006$ by Wilcoxon test). Positive family history also was positively associated with elevated PSA defined as ≥4 ng/mL ($p = 0.007$, Table 1).

PhIP and PSA. As noted above estimated PhIP intake had a highly skewed distribution, so association between PhIP intake and highly elevated PSA (≥20 ng/mL) status was investigated using PhIP-intake bin boundaries defined by the 50th, 70th, and 85th percentile values of the empirical intake distribution. PhIP-intake level was positively associated with highly elevated PSA status, when comparing those in the highest 15% vs. the lowest 50% of PhIP consumption, with or without single-variable adjustment for father/brother family history of PC, saturated fat intake, or total energy (corresponding p-values for trend: $p_{trend} = 0.01$ for saturated-fat adjustment, $p_{trend} \leq 0.003$ for all other tests) (Table 3). All highly elevated PhIP measures occurred in men within a fairly narrow age range (55 to 65 years old). In analyses limited to men age ≥50 years, men in the highest 15% compared to those in the bottom 50% of estimated PhIP intake had a conditional maximum-likelihood odds ratio of 31 (95% CI: 3.1 - 690) for having a highly elevated PSA ($p_{trend} = 0.0002$). For this age group, adjustment for family history of PC, saturated fat intake, or total energy yielded identical odds-ratio estimates, and only slightly greater p_{trend} estimates (data not shown).

A similar pattern of positive association was observed between estimated PhIP intake and men with highly elevated PC risk defined as either PSA ≥20 ng/mL or a "suspicious" abnormal DRE result leading to medical referral (data not shown). As expected, men with mildly elevated PSA (≥4.0 ng/mL) were about 4-times more likely to have had a suspicious DRE than did participants with PSA <4.0 ng/mL, although this difference did not rule out chance (p = 0.07, Fisher exact test). Suspicious DRE results were not obtained for any participant with a PSA measure ≥20 ng/mL. PSA level categorized either in quintiles or {50, 70, 85}th percentile intervals) was not associated with saturated fat intake, total energy intake, or body mass index, with or without adjustment for PhIP intake (p_{trend} >0.10 for each).

Discussion

These interim data are consistent with the hypothesis that estimated dietary exposure to PhIP is related to screening indicators of PC risk such as elevated PSA levels or suspicious DRE results among African American men. Although this conclusion remains preliminary due to the small number of men in this prospective study to date, it is supported by the consistency of the pattern of results observed and their level of statistical significance. We observed a positive association between elevated PSA and a father/brother history of PC, which is consistent with other studies in the literature that have linked such history to elevated PC risk[45-48], and reflects the integrity of the study design.

A potential specific link between PhIP intake and the elevated risk of PC experienced by African American compared to Caucasian men is suggested by relatively greater levels of PhIP and its metabolites detected in urine sampled from the two groups.[121] However, urine analyses only provide a measure of PhIP exposure within 12-24 hours prior to sampling.[122] Such a link is supported by studies using meat- and cooking-method-specific HA-concentration estimates from multi-laboratory sets of experimental cooking data.[113-115] These studies estimated intake of PhIP and other HAs among >20,000 U.S. participants (including >3,000 African Americans) in the nationwide, stratified, random-sample *U.S. Continuing Survey of Food Intake by Individuals* (CSFII).[124] Analyzed by age-, sex, and race/ethnicity, these HA-exposure assessments showed that African American men consumed on average at least twice (and boys through age 15 about three times) as much PhIP (and total HA) per kg body weight per day as did corresponding U.S. Caucasians, and that for both groups there was a small, positive correlation between estimated mean intake of PhIP and of saturated or total fat.[113-114]

No PhIP-related PC associations were reported in a study of 317 PC cases and 480 age-matched controls that compared HA intake (primarily from cooked lamb) and PC risk in New Zealand men.[78] They did report an association between PC risk and consumption of beefsteak by the higher cooking-doneness category (OR [95% CI]

= 1.8 [1.1 – 3.0], 2-sided p_{trend}=0.008). The relevance of this finding to potential associations between PC and dietary HA or PhIP intake in the U.S. for African American men is unknown. The incidence of PC in New Zealand is only half that of Caucasian men in the U.S.[44] Average meat (primarily lamb) consumption by participants (~150 g/d) was well below that of U.S. Caucasian men (260 g/d) and even further below that of African American men (304 g/d).[67 [Table 10A]] Moreover, the estimated minimum PhIP intake (224 ng/d) in the *top* exposure quartile of the New Zealand men[78] was well below the estimated *average* PhIP intake by Caucasian men (390 ng/d) and that of African American men (600 ng/d).[113] This difference reflects dissimilar meat consumption and cooking patterns in these two groups. Assuming a linear dose-response, extrapolation from elevated OR estimates ≥2.2 obtained in the present study down to PhIP intakes as low as those in the New Zealand study would require that study to have detected an OR as low as ~1.2 with 95% confidence at 80% statistical power, whereas only OR values ≥1.6 were so detectable in that study.[124] Alternatively, the lack of a PhIP-related association with PC risk in the New Zealand case-control study could reflect that men from that study (or perhaps all Caucasian men) are, for genetic and/or environmental reasons, less susceptible to the effects of this risk factor than are the African American participants of our clinic-based study.

Ideally, a prospective study accumulates good diagnostic data and data on exposure- or treatment-related variables of interest. One key limitation of this study is that, despite ongoing work to obtain corresponding follow-up diagnostic data, a PC diagnosis is not yet available for all participants who received either positive or highly elevated PC screening results or who received a "suspicious" DRE leading to medical referral. Compared to a case-control design, the prospective design used in this ongoing study has the advantage of being double blind, insofar as PC screening results are not known by the participant or by study investigators until after each participant has provided dietary survey data. This design eliminates potential bias in participants' self-reported cooking preferences that may be associated with knowledge of PC-screening results or PC status. This is important in view of evidence that prior knowledge of cancer-related status may affect dietary recall and so induce significant differential misclassification.[125]

A second key limitation was the lack of any biomarker generally considered reliable enough to assess chronic PhIP exposures. This required our study, like previous similar studies of HA-related cancer risks, to rely instead on FFQ data. Despite their tendency to slightly underestimate intakes, relative to estimates made using more accurate but also more inconvenient and expensive multiple 24-hour food diaries, FFQs have been widely used as the method of choice to assess long-term nutritional exposures, and to estimate specific disease risks in relation to such exposures.[126] Data from self-administered Block dietary FFQs, in particular, were shown to be effective at estimating intakes of each of four major meat-related nutrients (protein, total fat, saturated fat, and cholesterol) to

within <15% of corresponding estimates obtained using 24-hour recall diaries among 226 men who participated in the 1997-1998 "Eating at America's Table" study.[127] A self-administered meat-specific FFQ including questions on doneness and cooking-method preferences (similar to the meat-specific FFQ used in the present study) was more recently compared directly to data from 24-hour recall diaries.[112] In that study, the FFQ method was shown to underestimate PhIP intakes, but also to classify >60% of individual intakes of each of two HAs (including PhIP) to the same or adjacent quintiles, and to correctly classify lowest vs. highest quintiles for >94% of such intakes.[112] That study concluded that observed levels of FFQ-associated PhIP intake misclassification would tend to cause underestimation of true relative risks associated with increased PhIP intake estimated from self-administered FFQ data.[112] Rates of misclassification of PhIP and other dietary factors were likely reduced in the present study because our FFQs were administered in person by trained interviewers.

In conclusion, we applied methods to estimate HA concentrations in cooked meats based on individually expressed data on meat-specific intake, cooking method and doneness preference to estimate daily PhIP intake. We observed these intake estimates to be positively associated with screening indicators of highly elevated PC risk in a prospective clinic-based PC screening study with nearly 400 African American men in the East Bay Oakland, CA area. The observed positive association, even more significant among men 50 to 70 years of age, remained statistically significant after adjustments for saturated fat intake, total energy intake and self-reported father/brother history of PC. We will continue to accrue participants in this study, expand the screening indicators used to predict PC status, and assess whether the observed PhIP-related association pertains to incident PC disease.

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Table 1. Baseline characteristics of 392 participants in a San Francisco East Bay, CA study on diet and prostate cancer screening.

Variable[a]	Value(s)	*n*	Mean
Age (y)	39 to 50	115	47
	51 to 60	216	55
	61 to 70	61	64
	All	392	54
Weight (kg)	54 to 163	392	86[b]
BMI (kg cm^{-2})	<30	317	25
	≥30	75	34
	18 to 46	392	27
PSA (ng/mL)	< 2	328	0.79
	2 to <4	42	3
	4 to <10	10	6
	10 to <20	7	14
	≥20	5	47
		PSA<4	PSA≥4[c]
DRE	Normal[c]	233	15
	BPH[c]	108	4
	Suspicious[c]	12	3
Family history[d]	No	333	15
	Yes	37	7

[a] BMI = body mass index; DRE = digital rectal exam; PSA = prostate-specific antigen.

[b] Weight median (interquartile range) = 84 (75 to 95) kg.

[c] BPH = benign prostatic hyperplasia; Normal = no urinary, BPH or other symptoms; Suspicious = abnormal DRE result leading to medical referral. Each abnormal PSA result (≥4.0 ng/mL) triggered a medical follow-up referral.

[d] Family history = self reported brother(s) and/or father diagnosed with prostate cancer. Association of positive family history with PSA ≥ 4 ng/mL; Fisher exact test (p = 0.007).

Table 2. Summary of meat-specific PhIP intake for 392 study participants in an Oakland, CA study on diet and prostate screening.

	Meat type						
	Chicken	Burger	Beef	Pork	Fish	Bacon	All
Mean[a]	10.3	2.5	2.4	0.3	0.4	<0.1	16.7
SD[a]	13.	3.7	4.3	0.6	1.0	0.1	17.
SDM[a]	0.6	0.2	0.2	0.03	0.05	0.01	0.8
CVM%	6.2	7.4	9.1	8.2	15.	30.	5.1
%All	61.	15.	14.	2.2	2.2	0.1	100.

[a] Values listed are in ng/kg-day; arithmetic mean values are shown to a value of 0.1 ng/kg-day. Meats listed exclude those rarely used or meats that account for relatively little HA, such as lamb and organ meats. The meat components of food mixtures were excluded from those listed individually, but are included in the "All" category. SD = standard deviation. SDM = SD of the mean, CVM% = 100% x (SDM/mean).

Table 3. Association of PhIP intake with elevated Prostate-Specific Antigen (PSA) ≥20 ng/mL among 392 African American men, Oakland, CA.

Adjustment or stratification[a]	Ave. PhIP intake[a] PR (ng kg^{-1} d^{-1})	PSA ≥20 ng/mL[b] *m*	*n*	OR[c]	95% CI[c]	p_{trend}[c]
All data						
0-50:	4.8	0	196	1		
>50-70:	14.6	1	78	7.6	–	
>70-85:	25.5	1	59	10.	–	
>85-100:	49.7	3	59	24.	2.2 - 540	0.002
Adj. for FH						
(same as above)		(*m*, *n*, ORs, LCLs and UCLs same as for All data)				0.003
FH –						
0-50:	4.8	0	174	1		
>50-70:	14.6	1	69	7.6	–	
>70-85:	25.5	1	53	9.8	–	
>85-100:	49.7	1	52	10.	–	0.10
FH +						
0-50:	4.8	0	22	1		
>50-70:	14.6	0	9	–	–	
>70-85:	25.5	0	6	–	–	
>85-100:	49.7	2	7	18.	1.4 - 540	0.002
Adj. for SatFat						
0-50:	4.8	0	196	1		
>50-70:	14.6	1	78	3.4	–	
>70-85:	25.5	1	59	4.1	–	
>85-100:	49.7	3	59	24.	2.2 - 540	0.01
Adj. for KCAL						
0-50:	4.8	0	196	1		
>50-70:	14.6	1	78	14.	–	
>70-85:	25.5	1	59	4.3	–	
>85-100:	49.7	3	59	24.	2.2 - 540	0.003

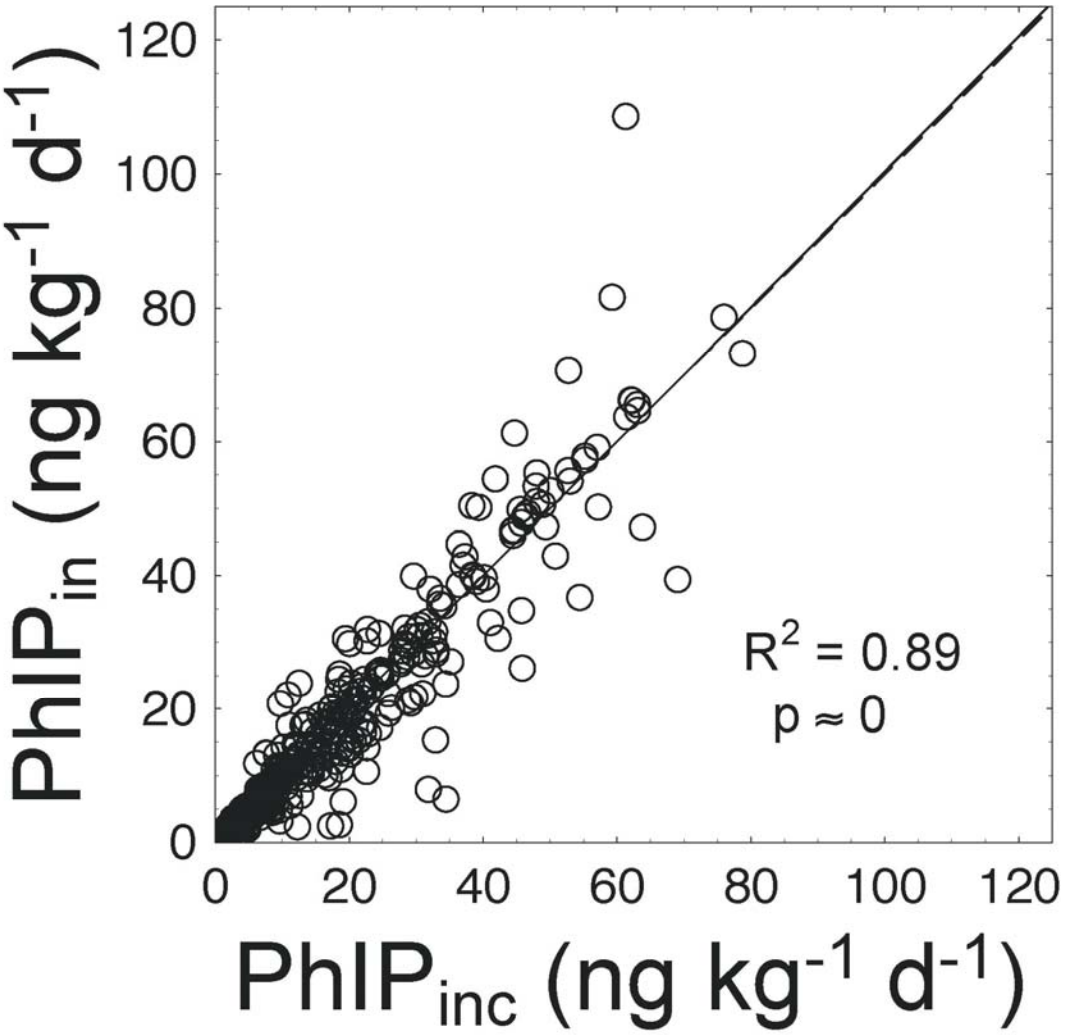
[a] PR = percentile range (PR and corresponding mean PhIP-intake values pertain to all 392 participants combined); FH = family history of prostate cancer,self reported father or brother diagnosed; SatFat = daily saturated fat intake per kg body weight; KCAL = total energy intake per kg body weight. Trend analyses adjusting for SatFat or KCAL were each done using the adjustment variable dichotomized at its median value. All men were ≤70 years old

[b] *m* = number with PSA ≥ 20 ng/mL among total n participants included in the analysis

[c] OR = maximum likelihood odds ratio estimate; CI = confidence interval (2-tail), omitted if interval includes 1; p_{trend} = p-value for chi-square test of linear, or (as indicated) adjusted linear, trend.

Figure Legend

Figure 1. PhIP intake estimated using individual-level doneness-preference data (Y-axis, $PhIP_{in}$), compared with intake estimated using all individuals combined with their meat-cooking-method-specific average levels of preferred doneness (X-axis, $PhIP_{inc}$). The linear fit of the data shown (dashed line) has an estimated (and corresponding 95% CI) intercept of 0.58 (–1.4 - 0.23) and slope of 1.01 (0.974 - 1.045); an identity relation (solid line) is shown for comparison.



Prostate-specific antigen levels and dietary PhIP in African Americans: A prospective clinic-based study

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Heterocyclic amines (HAs)—potent mutagens formed as red meat, chicken or fish cooks—cause cancer at multiple sites (including rat prostate) in rodent bioassays, and have been linked to elevated human risk of colon and other cancers. Compared to white men in the U.S., African American (AA) men have about twice the prostate cancer (PC) incidence and about twice the daily intake of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the predominant HA in the U.S. diet. To investigate the hypothesis that dietary PhIP exposure increases PC risk, an ongoing prospective clinic-based study has compared PC screening outcomes with survey-based estimates of dietary PhIP intake by 40- to 70-yearold AA men in the Oakland, CA, area. Participants with no prior PC diagnosis, recruited through a cancer education center/screening clinic, complete food-frequency and meat cooking/consumption questionnaires, and have a prostate-specific antigen (PSA) test and digital-rectal exam. Preliminary results for **562 participants** indicate that mean (±1 SD) daily intake of PhIP, the major HA found in cooked meats, in this group is 19 (±24) ng kg-1 d-1, which is ~2-fold (and ~3-fold) greater than a national estimate of mean PhIP intake for AA (and white U.S.) men of similar age. In the present study, estimated PhIP intakes were found to be attributable mostly (65%) to chicken and positively associated ($R2 = 0.25$, $p \sim 0$) with estimated saturated fat (SF) intake (a previously hypothesized environmental PC-risk factor). An odds ratio, OR, (and maximum-likelihood 95% confidence limits) of **23.6 (2.20, 533.)** for PSA ≥20 ng/mL was observed for those in the highest 15% compared to the lower 50% of estimated daily PhIP intakes (≥32 vs. ≤4.8 ng kg-1 d-1), with a p-value (ptrend) of 0.0023 for a chi-square test for trend done across three (including these two) PhIP-intake groups (extended Fisher exact test p-value = 0.0063). This positive trend persisted after separate adjustments for self-reported family (brother or father) history of PC (FH), SF intake, and energy intake (ptrend = 0.0024, 0.012, and 0.0032, respectively). PSA measures were found (by Kolmogorov 2-sample tests) to be significantly higher in AA men reporting a positive FH in this study ($p = 0.007$), particularly for those among the highest PSA-measure quartile in each FH group ($p < 0.0002$). These preliminary results are consistent with a positive association between PhIP intake and highly elevated PSA levels, supporting the hypothesis that diet and food preparation interventions may help reduce PC risk in AA and perhaps other groups.

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Elevated Prostate-Specific Antigen in African American Men with High Meat-Carcinogen Intake: a ProsGarretpective Clinic-Based Study

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Heterocyclic amines (HAs)-potent mutagens formed as red meat, chicken or fish cooks-cause cancer at multiple sites (including rat prostate) in rodent bioassays, and have been linked to elevated human risk of colon and other cancers. Compared to white men in the U.S., African American (AA) men have about twice the prostate cancer (PC) incidence and about twice the daily intake of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the predominant HA in the U.S. diet. To investigate the hypothesis that dietary PhIP exposure increases PC risk, a clinic-based study compared PC-screening outcomes with survey-based estimates of dietary PhIP intake by 40- to 70- year-old AA men in the Oakland, CA, area. Participants with no prior PC diagnosis, recruited through a cancer education center/screening clinic, complete food-frequency and meat-cooking/intake questionnaires, and have a prostate-specific antigen (PSA) test, a determination of % free (unbound) PSA (%fPSA), and digital-rectal exam (DRE). Results for **702 participants** indicate that mean (±1 SD) daily intake of PhIP, the major HA found in cooked meats, in this group is 22 (±29) ng kg^{-1} d^{-1}, which is ~2-fold (and ~3-fold) greater than a national estimate of mean PhIP intake for AA (and white U.S.) men of similar age. Estimated PhIP intakes were attributable mostly (68%) to chicken. An odds ratio, OR, (and maximum-likelihood 95% confidence limits) of **10. (2.9, 58)** for men at high PC risk was observed in the highest quartile compared to the lowest half of estimated daily PhIP intakes (328.0 vs. 213.6 ng kg-1 d-1) (ptrend = 0.00005), where high PC risk was defined as either a highly elevated PSA ≥20 ng/mL with %fPSA ≤ 25% or a “suspicious” DRE exam result. Interestingly, this trend was evident only in the group with self-reported family (brother or father) history of PC (extended Fisher-exact p = 0.00003), and not those without such history (ptrend = 0.44). The significant trends persisted after adjusting for SF intake or energy intake. These results are consistent with a positive association between PhIP intake and highly elevated PSA levels, supporting the hypothesis that diet and food preparation interventions may help reduce PC risk in AA and perhaps other groups.

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Diet, Genetics, Education, and Prostate Cancer Screening in an Outreach Clinic for African-American Men

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Background: African American (AA) men in the United States experience a disproportionate health burden due to prostate cancer (PC) and other chronic diseases. Differences in socioeconomic status (SES), screening behavior, culture, lifestyle habits, genetics, and access to medical care may contribute to this disparity. Prior work by our collaborative group indicates that AA men consume 2-3 times the amount of the dietary carcinogen PhIP (2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine) compared to Caucasians and that PhIP levels in AAs correlate strongly with prostate-specific antigen (PSA) level. Our research group also has a history of successfully implementing a PC screening and outreach program to lower-income AA men. Through that work, we have identified the need for improved outreach education regarding healthy eating and grocery shopping practices. It is our goal to address both these scientific and community needs in the proposed study.

Hypothesis 1. Diet, PhIP, and PC - We hypothesize that high intake of PhIP will be positively associated with PC risk indicators, while greater consumption of vegetables and antioxidants will be inversely associated with PC risk indicators and these dietary factors will modify the association of PhIP with PC in 1000 AA men.
Hypothesis 2: Gene variants, diet, PhIP, and PC - We hypothesize that gene variants (*UGT1A1, SULT1A1, CYP1A2, CYP1B1 GST* and *SOD2)* will modify the associations between these dietary factors and PC risk.
Hypothesis 3: SES, diet, and PC - We hypothesize that lower socio-economic status (SES), and diet/disease knowledge will be inversely correlated with healthy dietary practices and positively correlated with PC risk.
Outreach Objective: We will develop a locally-directed, culturally-sensitive educational guide to healthy eating and grocery shopping for PC and chronic disease prevention; disseminate this via peer counseling and outreach programs; and track the usage of this tool for 12 months. We hypothesize that this will facilitate adherence to healthy eating recommendations in this community of AA men.

Aim 1. Diet, PhIP, and PC risk - We will examine associations between intake of cruciferous vegetables, other vegetables/fruits, antioxidants, meats, and fish; exposure to PhIP, and PC risk indictors (e.g. PSA, %fPSA, DRE, EPCA2) among 1000 AA men. Linkage to the California Cancer Registry will also be conducted to examine associations of these dietary exposures with subsequent PC diagnosis.
Aim 2. Gene variants, diet, PhIP, and PC - We will examine modifying effects of selected gene variants (*UGT1A1, SULT1A1, CYP1A2, CYP1A1, GST* family*, SOD2*) on the diet-PC associations in Aim 1.
Aim 3: SES, diet, and PC - We will use self-reported zip code and data from the 2000 U.S. Census to obtain information on SES in underserved AA men. We will collect self-reported SES data via in-person interview from 300 newly recruited AA men to validate this area-based measure. We will examine correlations between SES and dietary knowledge and habits, other lifestyle practices, and PC risk.
Aim 4: Improving Outreach – We will develop a culturally-sensitive and practical Healthy Living and Shopping Guide for PC and chronic disease prevention to disseminate during screening and other outreach programs. We will collaborate with the American Cancer Society Speakers' Bureau to train volunteers recruited during screening to disseminate our guide and its content throughout the community.

Study Design: This is a prospective cohort study of 1000 AA men who attend a clinic-based screening and outreach program in Oakland, CA. 700 men are already enrolled and we propose to enroll 300 additional AA men.

Impact: Our study will have a direct impact on reducing health disparities in PC by providing PSA screening, medical outreach, and healthy living education to a high risk, under-served population of AA men, many of whom are likely uninsured and of low SES. Our study's results will help fill a critical gap in the scientific knowledge regarding the role of diet and genetics in PC development in AA men.

Innovation: This study is innovative with regards to its excellent translational design, novel outreach/education approach, creation of a self-sustaining outreach educational tool, examination of a new biomarker for PC screening, and unique focus on high-priority PhIP-diet-gene interactions in a population of under-served AA men.

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ORIGINAL ARICLE

Development of a meat frequency questionnaire for use in diet and cancer studies

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***Key words*:** meat, food frequency questionnaire, heterocyclic amines

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Abbreviations (CAS #): HA = heterocyclic amine, PC = prostate cancer, PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (105650-23-5).

Abstract

Objective: To develop a meat frequency questionnaire (MFQ) to assess dietary heterocyclic amine (HCA) intake. The MFQ is designed to obtain information on meat types, cooking methods and doneness preferences that predict HCA concentrations in different meats.

Design: Total and specific meat intakes were determined by a standard food frequency questionnaire (FFQ) and compared with that determined by the MFQ.

Subjects/Setting: 314 African-American males participating in a clinic-based study of prostate disease and HCA intake were administered the two questionnaires in a cancer education center prior to undergoing screening evaluations for prostate disease.

Main outcome measures: HCA vs. total meat intake was assessed using the MFQ vs. FFQ, respectively. Specific meat items included in the MFQ were evaluated as factors potentially explaining discrepancies in meat intake estimated using the two questionnaires. Seasonal variation in meat intake was also examined.

Statistical Analysis: Correlation coefficients for intake of total meat and individual meat groups determined by FFQ vs. MFQ were calculated. Seasonal differences in meat and estimated HCA intakes were evaluated by t-test, adjusted for multiple comparisons.

Results: Meat intakes determined by the two questionnaires were well correlated (Pearson r = 0.69); however, total meat assessed by the MFQ exceeded total meat assessed by the FFQ in 30% of participants. Total energy and intake of HCA-associated meat was greatest when the MFQ was administered during winter months.

Conclusions: The HCA MFQ provided a fractional measure of total meat intake and identified specific HCA-containing meat items underreported in a standard FFQ.

***Key words*:** meat, dietary questionnaire, heterocyclic amines, PhIP, cancer

Introduction

HCAs are formed in meat, chicken and fish through reactions of natural constituents of muscle tissue (amino acids, sugars, nucleic acids and creatinine) that occur under certain cooking conditions (1; 2). Meat type, cooking method, and cooking duration and temperature are among factors known to influence the extent to which HCAs form in cooked meats (3-7). HCAs are relatively potent mutagens in the Ames assay, are effective carcinogens in laboratory mice and rats, and their intake has been associated with increased cancer risk in humans (8-10). Estimates of average daily intake of HCA (primarily phenylimidazo[4,5-*b*]pyridine, or PhIP) in the U.S. include 6.3 ng/kg/day (11) and 13 to 18 ng/kg/day (12).

Studies of the health effects of HCA intake have relied on various methods of dietary assessment to determine intake of meat (beef and pork), chicken and fish, the primary sources of HCA in the diet. The most common method has been the food frequency questionnaire (FFQ), a recall of the frequency and portion sizes of all foods consumed over the past 12 months. Since HCA levels in cooked meats are influenced by meat type and cooking conditions (2), FFQs have been supplemented with questions about cooking method and doneness preference. However, widely-used FFQs group meat type and cooking method into general questions about meat intake and thus may not obtain the detailed information needed to accurately assess HCA intake. In addition, seasonal differences in the consumption of certain foods and the use of HCA-forming cooking methods (barbeque/grilling) may lead to differential recall of meat intake when assessed with a general commonly-used FFQ administered at different times of the year.

To supplement dietary survey instruments that do not account for HCA-related factors, we developed a meat frequency questionnaire (MFQ) that focuses exclusively on those meat types, cooking methods and doneness preferences that are associated with HCA intake. The MFQ elicits data on which specific meat types are consumed and how these are typically cooked. In a preliminary assessment of the MFQ, we compared estimated intakes of HCA-associated cooked meat, chicken and fish obtained using the MFQ to estimates of total intake of meat, chicken and fish assessed with a general, commonly-used FFQ. We wanted to examine the consistency of the MFQ to report meat intake as a fraction of that reported by the FFQ. We also sought to evaluate the intake of specific meat items in the MFQ that are not specifically queried in the FFQ.

Methods *Survey Group*: Using an Lawrence Livermore National Laboratory IRB-approved human subjects research protocol, African-American males from underserved communities in the Oakland, CA, area were solicited for participation in a study of the possible association between prostate disease and HCA intake. African Americans, who have the highest rates of prostate cancer worldwide, have been shown to have the highest daily HCA intake of any U.S. ethnic group (13), which in view of

evidence for HCA genotoxicity and carcinogenicity, suggests that HCA intake may partially explain the racial/ethnic disparity in prostate cancer risk. Selection criteria for the study were non-vegetarian males between the ages of 40 and 70 with no prior history of prostate cancer. For the study, 314 participants were provided $30.00 to complete the two questionnaires and to receive a prostate specific antigen blood test and digital rectal exam. Questionnaires were administered sequentially in one-on-one interviews at the cancer education center from September, 2002 to April, 2004.

Diet Questionnaires: The Block Brief-2000 Food Questionnaire (Block Dietary Data Systems, Berkeley, CA) was used to obtain estimates of total energy intake to assess reporting accuracy and measures of total meat intake. This FFQ consists of 87 questions about the consumption of foods, beverages and vitamins compiled from previous versions of the NCI Health Habits and History Questionnaire (14, 15) and is designed to capture 80% of energy intake. The Brief-2000 FFQ condenses the 22 meat questions from a full-length FFQ to 12 meat questions by grouping meat categories into fewer questions and excluding rarely consumed meat parts. The FFQ was used to obtain summary data to evaluate the reporting accuracy of the respondents (energy intake), intake of potential confounders of prostate disease (saturated fat) and total meat intake over the past 12 months. Responses from 10 questions on beef, meat mixtures, pork, chicken and fish were used to estimate total meat intake. Consumption frequency (how often per year, month, week or day) and portion size determined from the selection of photographs depicting 4 quantities of food or 4 portion size descriptions (e.g., how many pieces; 1/8 to 3/4 lb) were obtained for each item. The FFQ combines different meat cuts and cooking methods into single questions (e.g., intake of non-fried chicken is queried by the following question: "How often in the past year did you eat chicken or turkey not fried, such as baked, grilled, or in sandwiches"). Responses from the FFQ were converted to energy (kcal d^{-1}) and saturated fat (g d^{-1}) intakes by Block Dietary Data Systems using proprietary nutrition analysis programs.

The MFQ ascertains the intake over the past 12 months of HCA-associated cooked meat, chicken and fish. Questions on the MFQ ask only about meat and meat cuts cooked exclusively by HCA-forming methods (e.g. steak and ribs, assumed to be either grilled, fried or broiled) or specific meat type/cooking method combinations for those meats capable of being cooked by either HCA-forming or HCA-nonforming methods (e.g.,"How often did you eat chicken: pan fried, not deep fried?"). Consumption frequency (how often per year, month, week or day) and portion size (determined by asking participants to select from four color photographs of meat models [Nasco, Modesto, CA] arranged from single to multiple servings) are obtained for each item. Another series of questions assess: where specific meat items are typically consumed (home, fast-food restaurant, other type restaurant), typical meat cooking methods (grilled/BBQ, broiled, fried, or other), and doneness level of items cooked (rare/medium, well done, very well done, extra well done). Typical doneness preferences are also assessed via selection among

a series of color photographs depicting specific meats cooked to levels of increasing doneness. Hamburger intake was assessed with the MFQ by including a question about the consumption of meat loaf (assumed to be baked, a method that forms little if any HCAs) on the MFQ and deducting the response from the FFQ question about consumption of hamburgers/meat loaf.

For the interview, the FFQ was administered first to participants followed by the MFQ. The FFQ and MFQ were scanned for data acquisition yielding data in ASCII format for analysis. Estimated total energy (kcal d^{-1}) was included in each FFQ data file. For both questionnaires, daily intakes of beef, pork, chicken and fish for each participant were calculated by multiplying the reported portion size by the annual consumption frequency (days consumed per year/365) for each meat item and summing items within each meat group. The FFQ also asked about the consumption of meat mixtures and tacos, burritos, enchilada, and tamales. Daily meat intakes from these food items were multiplied by the percentage of meat in these items as reported in the Recipe Database of the USDA 1994-1996 Continuing Survey of Food Intakes by Individuals (16).

Comparison of Intake Estimates: Daily mean values for the intake of energy and total meat obtained with the FFQ were compared with values reported for African-American men ages 40-70 in two national dietary surveys, the 1994-1996 Continuing Survey of Food Intake by Individuals (17) and the 1988-94 National Health and Nutrition Examination Survey (18). These surveys are both dietary recall surveys that utilize interviewers and food models to accurately ascertain food intake for two nonconsecutive 24 hour periods. Differences in total meat intake (TMI) obtained with the FFQ and MFQ were based on the following ratio: TMI_{MFQ}/TMI_{FFQ} where TMI_{MFQ} is total consumption of beef, pork, chicken and fish determined with the MFQ and TMI_{FFQ} is defined as total consumption of beef, meat mixtures, pork, chicken and fish determined with the FFQ, both in units of g meat d^{-1}. Finally, the ratio of estimated energy intake to estimated basal metabolic rate (both per unit lean body mass) was calculated for each participant using energy intakes (kcal d^{-1}) estimated by the FFQ and basal metabolic rates (BMR, in kilocalories per kilogram body weight) estimated using an algorithm (13) developed from data presented by Layton (19) and Schoeller and van Santer (20). The BMR algorithm utilized self-reported age and body weight (in kg) provided by the study participants.

Statistical Analysis: Pearson product-moment correlation coefficients for intake of total meat and individual meat groups determined by the FFQ and MFQ were calculated. Seasonal differences in food intakes were assessed by comparing mean intakes between participants completing the FFQ and MFQ during 3 summer months (June, July and September) and participants for all other months, and by comparing mean intakes between participants completing the FFQ and MFQ during the 3 winter months (November, December and February) with those in all other months. Seasonal comparisons were evaluated

by t-test or Welch's approximate t-tests in cases of nonhomogeneous variance (21). P-values with and without Hommel's Bonferroni-type adjustment for multiple independent tests are reported (22).

Results

Energy intake estimated by the FFQ exceeded that estimated in both national dietary recalls whereas daily meat consumption obtained with FFQ was less than that obtained in the CSFII survey (Table 1). The average of 314 ratios of reported energy intakes determined by the FFQs to estimated corresponding basal metabolic rates was not significantly different by t-test ($p = 0.12$) from the value of 1.6 expected for reference adults (13, Figure 1). The wide range of ratios obtained, however, suggests that underreporting and over-reporting of total energy intake occurred by roughly 20% and 10% of subjects, respectively, using questionnaires administered by direct interview to individuals in our study group. There was good correlation between intake of saturated fat and total energy ($r = 0.95$, ($p < 0.0001$).

Mean daily intakes (g/day) of beef and, pork determined by MFQ were comparable to those determined by FFQ, whereas mean daily intakes of chicken and fish estimated by MFQ were less than that determined by FFQ (Figure 2). The correlation of TMI_{MFQ} with TMI_{FFQ} was 0.69 ($p < 0.0001$), Figure 3). Correlations among meat-specific intakes estimated by FFQ vs. MFQ were 0.73, 0.52, 0.46 and 0.57 for beef, pork, chicken and fish, respectively (data not shown). Although for most participants TMI_{FFQ} exceeded TMI_{MFQ}, TMI_{MFQ} did exceed TMI_{FFQ} for 30% of participants. Factors that could explain higher estimated TMI using the MFQ were examined within individual meat groups. For beef, the MFQ-to-FFQ ratio of estimated beef consumption was positively associated with consumption of meat patties, a food item queried on the MFQ but not on the FFQ ($r = 0.58$, $p < 0.0001$). For chicken, this ratio was positively associated with the consumption of grilled chicken, a meat item explicitly queried on the MFQ but grouped in a FFQ chicken question ($r = 0.36$, $p < 0.0001$). Significant differences in energy and meat intakes were observed for summer and winter compared to the rest of the year. Specifically, energy and HCA-meat intakes were significantly lower in summer and higher in winter (Table 2). The greatest relative change observed to be seasonally related concerned mean PhIP intakes, which were observed to be elevated significantly by 52% in winter compared to non-winter months (adjusted $p = 0.02$) (Table 2).

Discussion

The assessment of the intake of HCAs requires information about cooking preferences and practices that is not typically obtained in the standard FFQ. In particular, meats cooked in specific ways (barbecue or pan-fried) can have particularly high HCA levels (5-7) so that FFQs that group meats into one question cannot account for HCA-containing meats that contribute to

exposure well beyond their gram-weight portion of the diet. The MFQ was designed to address the specific meat type/cooking method combinations that contribute most to HCA intake. To do so, the MFQ used more questions about meat intake than the typical FFQ which may result in higher estimates of meat intake simply due to greater recall or responsiveness of the questioned. Studies on questionnaire design have found that frequency of consumption is higher when foods are listed separately rather than grouped (23) and significantly greater hamburger consumption was reported when ground beef consumption was determined by two questions (one for hamburgers/cheeseburgers and one for ground beef in mixtures) rather than by one (both forms of ground beef in one question, 24). To evaluate whether the MFQ was over-estimating meat intake, we calculated the ratio of TMI_{MFQ} to TMI_{FFQ}, using a ratio of one as an indication that the MFQ was over-reporting meat intake. For this preliminary assessment, we used the FFQ as a measure of total dietary intake as no dietary records from our participants were available. Participant total energy calculated from the FFQ exceeded national estimates for this ethnic group obtained by dietary recall (Table 1). Mean food intakes for minority populations derived from FFQs have been shown in previous research to be higher than those estimated by diet recalls (25-28). Also, our participants were drawn from an underserved population and were surveyed for the entire year so socioeconomic and temporal factors may explain this difference. There are limitations to our approach however. The FFQ we used to assess total meat intake was designed to capture 80% of energy intake so the measure of total meat intake used in our assessment may be biased. Also, misreporting of food intake may have contributed to inaccurate measures of intake obtained with the FFQ, as evidenced by wide variability in estimated ratios of energy intake to basal metabolic rate (Figure 1). The good correlation of saturated fat with total energy does suggest that any misreporting of intake for fat-containing food groups is uniform so that the FFQ provides a consistent baseline for comparison with the MFQ within this group.

Mean daily intakes of chicken and fish estimated by MFQ were less than those estimated by the FFQ, but not so for beef. Beef is the meat type with the most grouped questions on the FFQ and the most individual questions on the MFQ. Studies on questionnaire design have found that frequency of consumption is higher when foods are listed separately rather than grouped (23) and significantly greater hamburger consumption was reported when ground beef consumption was determined by two questions (one for hamburgers/cheeseburgers and one for ground beef in mixtures) rather than by one (both forms of ground beef in one question, 24). The Block Brief 2000 is an abbreviated version of a more complete FFQ and is designed to capture 80% of actual intake (personal communication) which may explain some of the discrepancy between FFQ and MFQ beef intake estimates. We always administered the FFQ first to have the participant's total diet recall help inform him with the more

detailed recall required with the MFQ. With this design, we cannot rule out the possibility that on ordering effect is responsible for the FFQ-MFQ discrepancies

TMI_{FFQ} was expected to exceed TMI_{MFQ} as the FFQ was designed to capture total meat intake whereas the MFQ was designed to capture only intake of HCA-associated meat, a subset of the total. However, TMI_{MFQ} exceeded TMI_{FFQ} in a substantial number of cases and these cases were distributed evenly over the range of total meat intake. Some of these cases were attributable to reported consumption of exclusively HCA- associated meat on both surveys and questionnaire differences in portion size and consumption frequency resulted in higher TMI_{MFQ}. Systematic differences between the two questionnaires may also explain why TMI_{MFQ} exceeds TMI_{FFQ} for some participants. We included a question about meat patty consumption in the MFQ, a meat item consumed by African American males but not addressed in the FFQ. Meat patty intake correlated positively with the ratio of beef intake determined by MFQ to that determined by FFQ ($r = 0.58$) indicating that intake of this food item comprises a larger portion of meat consumption in those cases where MFQ meat intake exceeded FFQ meat intake. Likewise, intake of grilled and pan-fried chicken, food items that are grouped in a question on the FFQ but explicitly queried in the MFQ, correlated positively with the ratio of chicken intake determined by MFQ to that determined by FFQ ($r = 0.36$). A recent study found that an interview-administered FFQ substantially underreported the percentage of consumers of grilled/barbecued and pan-fried chicken (18 an 16%, respectively; 28) when compared with intake of these items determined by multiple food diaries. By explicitly asking about these two chicken items, the MFQ may more accurately capture total chicken intake. Accurate measurement of grilled and pan-fried chicken is important in HCA exposure assessment as chicken has been shown to have some of the highest HCA levels of all cooked meats (5) and to contribute the most to estimated U.S. daily HCA intakes (9; 13). We always administered the FFQ first to have the participant's total diet recall help inform him with the more detailed recall required with the MFQ. With this design, we cannot rule out the possibility that an ordering effect is responsible for the FFQ-MFQ discrepancies

Participants in this study reported the higher energy and meat intake when they completed the dietary questionnaires in the winter months, excluding January. This finding is marginal given our small sample size, the absence of multiple measures from the participants and incomplete sampling of all months throughout the year. Nonetheless, this finding is consistent with studies finding greater energy and meat intake in winter. Seasonal variability in excess of 5% was observed with an FFQ administered over the course of the 4 seasons though intake of fried chicken and fish were the only meats identified to vary by season in this study (30). Women reported higher energy intake in winter when administered an FFQ three times over a year (31), Israeli men

had greater energy intake and significantly greater meat intake in winter than in summer (32) and Shanghai women consumed 2.5% more meat in winter than summer (33). Finding high PhIP intake during the winter months was surprising given that the highest PhIP levels are found in barbecued meats, the intake of which is associated with summer. However, an exposure assessment based on national dietary data indicated that over 60% of African American male PhIP intake was attributable to the consumption of pan-fried chicken (13), a food item not associated with seasonal consumption. Further validation of these findings is warranted given the limitations of the study to assess seasonal variability.

The MFQ provided a fractional measure of total meat intake and identified specific HCA-associated meat items underreported in the FFQ. HCA concentrations in cooked meats can vary up to 10-fold depending on the meat type and how it is cooked so the omission of certain food items from an FFQ can contribute to underestimates of HCA intake well beyond their gram weight contribution to the overall diet. Meat patties and barbecued chicken were the two meat items that best correlated with the TMI_{MFQ}/TMI_{FFQ} ratio, indicating that intake of these meats was highest in those cases when the ratio exceeded one. These meat items are generally not included in (meat patties) nor listed exclusively (barbecued chicken) in standard FFQs. Consultation with a nutritional expert on the African-American diet (E. West, University of California) led us to include meat patty in the MFQ and our previous analysis identified barbecued chicken as the primary source of HCAs in the diet of African Americans (13). Thus, to estimate HCA intake in other populations with unique dietary habits and foods, different questions about unique meat intake may be needed in the MFQ.

Applications/Conclusions

An increasing number of studies of diet and cancer are assessing HCA intake due to evidence that intake of HCAs is associated with elevated cancer risk. Most FFQs do not contain the detailed questions needed to assess HCA intake so meat-specific questions like those we include in the MFQ must used to augment FFQs to estimate intake of HCAs. Development of an MFQ to augment an FFQ will depend on the degree to which the FFQ captures the specific information on meats, cooking method and doneness preference needed to calculate HCA intake. In most cases, the MFQ will need to acquire additional information on cooking method and doneness preference but specific meat types will also need to be queried as well when FFQ meat questions are grouped. Specific questions should be targeted at HCA meat items known to be in the diet of the surveyed group. When an MFQ is to be used, we recommend that the FFQ be administered first in its entirety followed by the MFQ with each meat group sequentially queried for the relevant information – cooking method, doneness preference and location consumed (home or restaurant). Generalized meat questions on the FFQ should be used for dietary and nutrient analysis after addition of intakes of specific meat items queried only in the MFQ.

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Table 1. Comparison of FFQ mean total meat and energy intake (n=314) with national estimates

Variable	FFQ[1]	CSFII[2]	NHANES[3]
Total Meat (g d^{-1})	198.5 ± 156.4	216.9	NR[4]
Total energy (kcal d^{-1})	2528 ± 1464	2014.	2190.

[1] Food frequency questionnaire (Block Brief 2000)

[2]1994-1996 Continuing Survey of Food Intakes by Individuals

[3]1988-94 National Health and Nutrition Examination Survey

[4]Not reported

Table 2. Comparison of energy, meat and PhIP[1] intakes by season.

Variable[2]	Period[2]	*n*	Mean (± SEM)[2]	p	p_{adj}[3]
Energy (Kcal/d)	Non-summer	238	2700 ± 100	7.1×10^{-5}	5.7×10^{-4}
	Summer	76	2030 ± 130		
$Meat_{MFQ}$ (g/Kg-d)	Non-summer	238	2.03 ± 0.12	0.011	0.044
	Summer	76	1.55 ± 0.14		
$Meat_{FFQ}$ (g/Kg-d)	Non-summer	238	2.39 ± 0.12	0.38	0.38
	Summer	76	2.18 ± 0.20		
PhIP (ng/Kg-d)	Non-summer	238	17.8 ± 1.3	0.38	0.38
	Summer	76	15.6 ± 2.0		
Energy (Kcal/d)	Non-winter	212	2360 ± 90.2	0.0039	0.022
	Winter	102	2910 ± 160		
$Meat_{MFQ}$ (g/Kg-d)	Non-winter	212	1.72 ± 0.11	0.0089	0.035
	Winter	102	2.31 ± 0.10		
$Meat_{FFQ}$ (g/Kg-d)	Non-winter	212	2.19 ± 0.12	0.055	0.17
	Winter	102	2.65 ± 0.21		
PhIP (ng/Kg-d)	Non-winter	212	14.8 ± 1.3	0.0034	0.020
	Winter	102	22.5 ± 2.0		

[1]PhIP = 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (105650-23-5)

[2] $Meat_{MFQ}$ = total intake of meat cooked by HCA-forming methods estimated using LLNL MFQ; $Meat_{FFQ}$ = total intake of meat estimated using Block Brief 2000 FFQ; "Summer" defined here as June/July/September (August unsampled), "Winter" as holiday months November/December/ February. SEM = standard error of the mean.

[3] Listed p-values are for corresponding t- or (in cases of nonhomogeneous variance) Welch's approximate t-tests (20). Listed p_{adj} values are Hommel's Bonferroni-type adjusted p-values that reflect performance of the 16 listed independent tests (22).

Figure Legends

Figure 1. Cumulative distribution of the ratio of energy intake to basal metabolic rate (*n* = 314). Mean value (±1 SEM) = 1.54 ± 0.05 (solid line and points) among subjects studied is compared to the value of 1.6 expected for reference adults (13).

Figure 2. Daily intake (g/day) of meat determined by FFQ and by MFQ. Top of box = average; bottom of box – median; bar = 1 standard deviation.

Figure 3. Correlation of total meat intake determined by FFQ (TMI_{FFQ}) and by MFQ (TMI_{MFQ}). Closed circles: $TMI_{FFQ} > TMI_{MFQ}$; open circles: $TMI_{FFQ} < TMI_{MFQ}$.

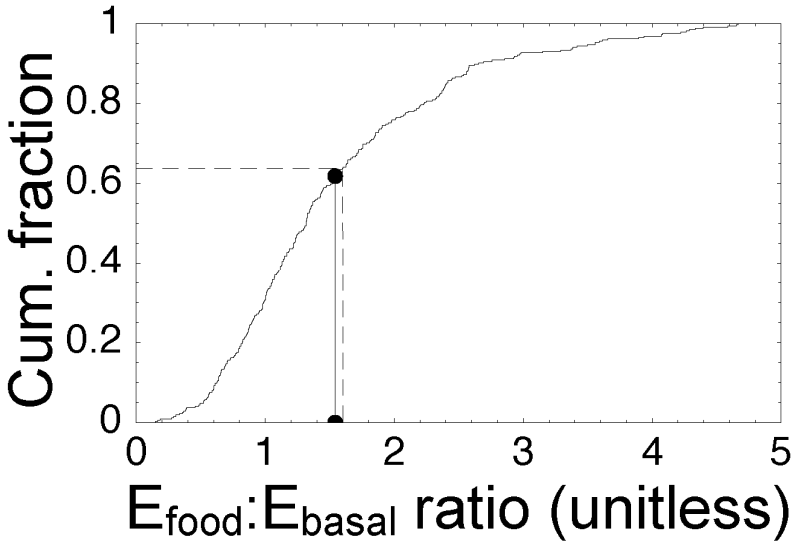
Cum. fraction
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
Efood:Ebasal ratio (unitless)

Figure 1

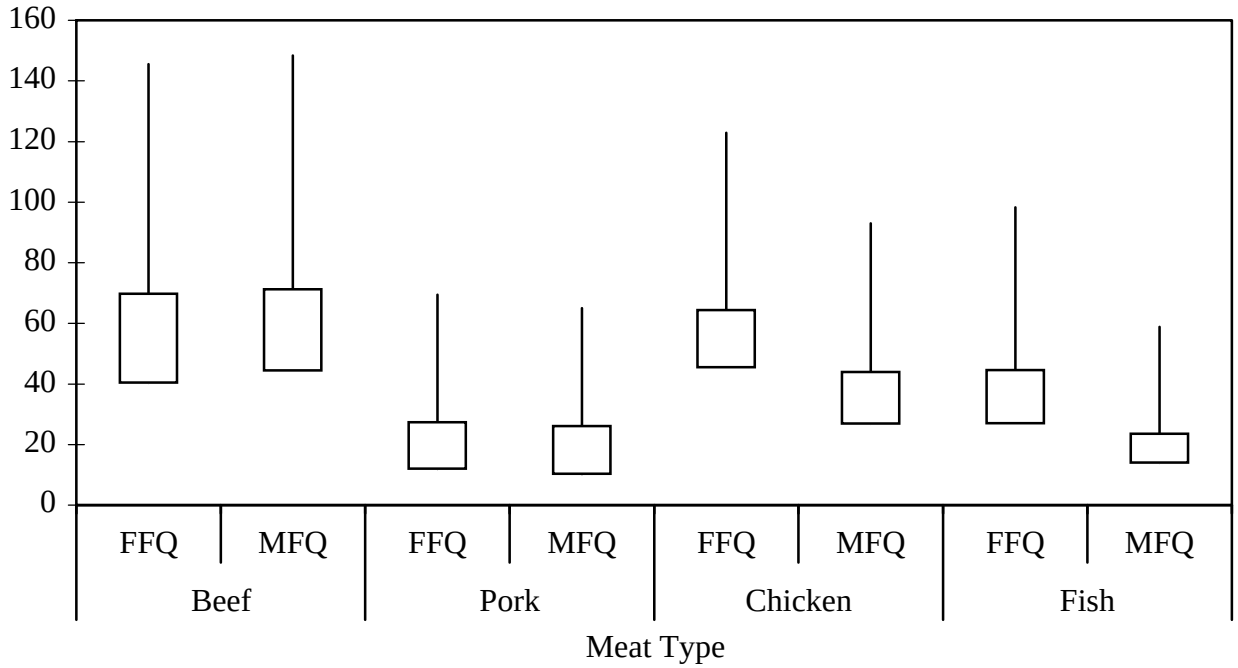
160
140
120
100
80
60
40
20
0
FFQ
MFQ
FFQ
MFQ
FFQ
MFQ
FFQ
MFQ
Beef
Pork
Chicken
Fish
Meat Type

Figure 2

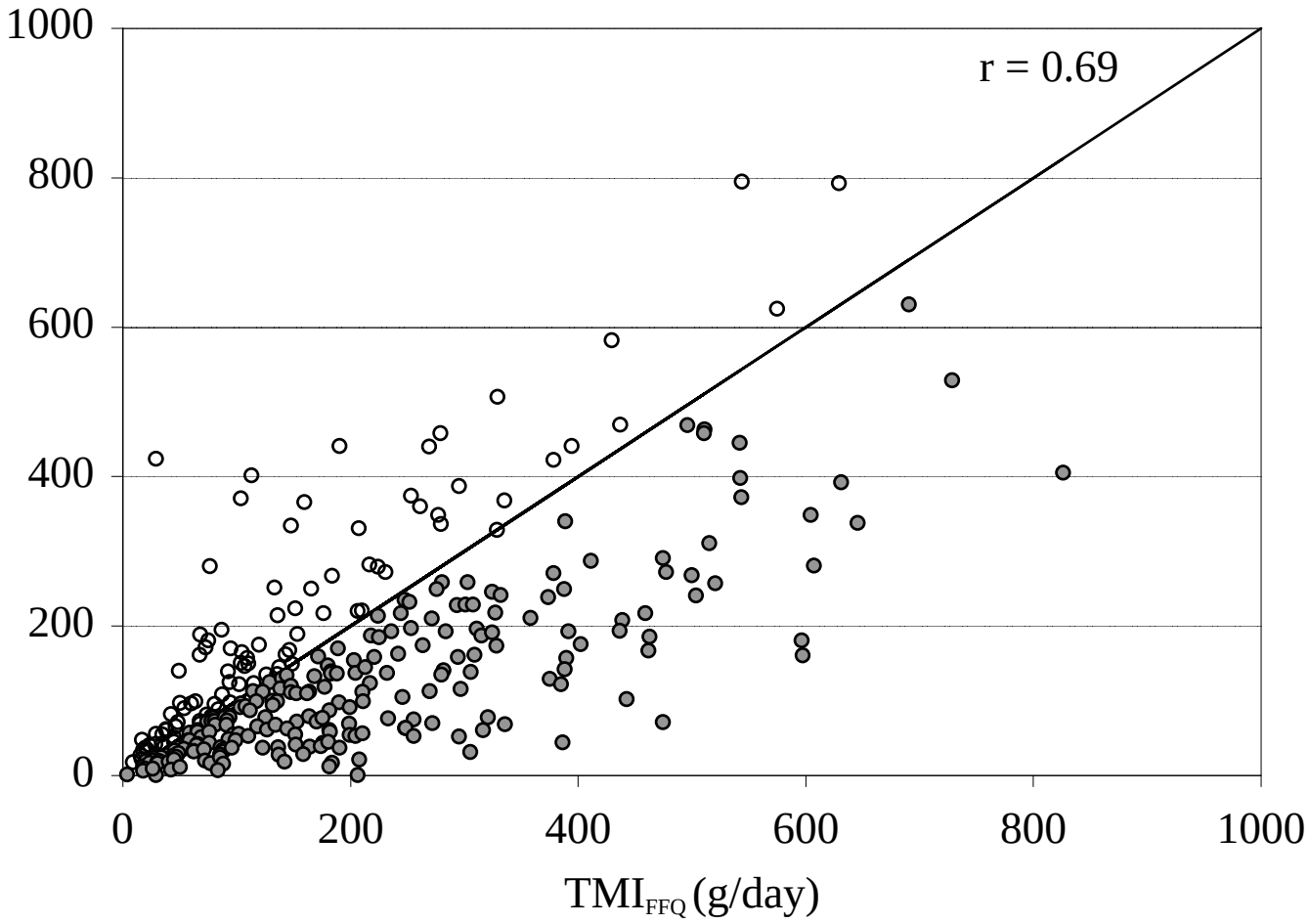

Figure 3